VOLUME 12 FEBRUARY, 1935 NUMBER 2

NATIONAL
RESEARCH COUNCIL
of CANADA

CANADIAN JOURNAL OF RESEARCH

CANADA

Published under the authority
of the
Chairman of the Committee of the
Privy Council on Scientific and Industrial Research

OTTAWA - CANADA

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The Canadian Journal of Research is published monthly by the National Research Council of Canada, Ottawa, to which address all correspondence should be directed.

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Canadian Journal *of* Research

Issued by THE NATIONAL RESEARCH COUNCIL OF CANADA

VOLUME 12 FEBRUARY, 1935 NUMBER 2

A STUDY OF VARIOUS FRACTIONS OF *BRUCELLA ABORTUS*

IV. RESIDUES FROM WHOLE FILTRATE AND AFTER REMOVAL OF ALCOHOLIC PRECIPITATE[1]

BY RONALD GWATKIN[2]

Abstract

Residue obtained by evaporation of a filtered suspension of *Brucella abortus* after removal of the alcoholic precipitate, and residue from whole filtrate, were less toxic for guinea pigs than the alcoholic precipitate. Residue of the supernatant liquid had less complement binding power than the precipitate and produced only a weak skin reaction in infected guinea pigs. Residue of whole filtrate, prepared from five-day cultures, was as active as the precipitate as a complement binding antigen, but material prepared from two- and three-day growth was weaker. Injections of these substances failed to protect guinea pigs against infection by eye with *Brucella abortus*.

In previous papers (1, 2, 3) the writer reported on a study of an alcoholic precipitate obtained from a filtrate of a suspension of *Br. abortus*. This paper deals with the product obtained by evaporation of the supernatant fluid, after removal of the alcoholic precipitate, and also with the residue obtained by evaporation of an untreated filtrate. *Br. abortus* was grown on liver agar for several days, washed off with water or salt solution, shaken with glass beads, centrifuged and filtered through a Mandler candle, as described in the first article of this series (1).

Residue Obtained from Supernatant Fluid after Removal of Alcoholic Precipitate

Obviously the addition of alcohol to a filtrate of a shaken suspension of *Br. abortus* would not remove all the material present. It was therefore decided to obtain the residue in the supernatant fluid by evaporation. Through the kindness of Dr. A. D. Barbour a constant flow vacuum still was set up by means of which the fluid could be evaporated off at low temperature. The first lot was evaporated by moderate heat above a hot plate but this was discarded in favor of low-temperature evaporation.

[1] *Manuscript received September 27, 1934.*
Contribution from the Department of Pathology and Bacteriology, Ontario Research Foundation, Toronto, Canada.

[2] *Research Fellow.*

Experiment 1. Simple evaporation of supernatant fluid. Three hundred cc. of the supernatant fluid of Lot 7 alcoholic precipitate was filtered through a candle and evaporated to dryness above a hot plate and finished *in vacuo* over phosphorus pentoxide. The yield was greater than that of alcoholic precipitate, 0.45 gm. being obtained from 300 cc. The powder was markedly hygroscopic and went into solution very readily in water.

Intraperitoneal injection of residue. Two guinea pigs were injected by the intraperitoneal route as follows: No. 1 received 0.04 gm. nd No. 2 was given 0.01 gm. of residue. Temperatures are shown in Table I.

TABLE I

TEMPERATURES OF GUINEA PIGS INJECTED WITH RESIDUE

	11:00 a.m.	1:30 p.m.	3:30 p.m.	5:00 p.m.	6:30 p.m.	9:00 a.m.	12:00 m.
No. 1	103.0	102.6	103.0	102.6	105.0	103.0	103.2
No. 2	103.0	102.4	101.6	101.2	102.4	102.2	102.6

It will be seen that the residue did not cause a drop in temperature of any extent. There was a passing rise in No. 1. The heart action was affected for a few hours but the animals were not otherwise affected.

Complement fixation test on precipitate and residue. A suspension of 0.01 gm. of each in 2.0 cc. saline was made and diluted 1 : 25, 1 : 100 and 1 : 500. The results of the test of residue compared with alcoholic precipitate from the same filtrate are given in Table II.

TABLE II

COMPLEMENT FIXATION TEST ON RESIDUE AND PRECIPITATE OF LOT 7

		1 : 25	1 : 50	1 : 100	1 : 250	1 : 500	1 : 1000	1 : 2500
Alcohol precipitate	1 : 25	4	4	2	2	0	0	0
Residue	1 : 25	4	4	4	4	4	3	0
Al. ppt.	1 : 100	4	4	4	4	0	0	0
Residue	1 : 100	1	1	1	0	0	0	0
Al. ppt.	1 : 500	4	4	4	4	4	0	0
Residue	1 : 500	0	0	0	0	0	0	0

4 = complete fixation. 3 = 75% fixation. 2 = 50% fixation. 1 = 25% fixation. 0 = no fixation.

The residue 1 : 25 was slightly higher than the 1 : 500 precipitate, but residue 1 : 100 showed only a trace of fixation and 1 : 500 gave none. The alcoholic precipitate was a much stronger antigen, but, as in all our tests, seemed to be inhibited in the lower dilutions.

Experiment 2. Lot 2 supernatant residue. The supernatant fluid from several lots was filtered through fine paper and run through the vacuum still. The temperature of the bath containing the distilling flask was kept at about 50° C. The fluid was reduced to 40 cc. and finished over a water bath between 70 and 80° C. and then *in vacuo* over phosphorus pentoxide. A yield of 8.3 gm. was obtained. Temperatures of injected guinea pigs went lower with this lot than with the first one, probably owing to larger dosage, but not as low as those of the precipitate animals. It proved to be fatal in doses of 0.1 cc. as four out of six injected guinea pigs died.

Protection test with residue. Six guinea pigs were given an intraperitoneal injection of 0.04 gm. This was the maximum injection given with the previous lot. It caused no ill effects and little change in temperature. The second injection of 0.08 gm. five days later caused more drop in temperature but was otherwise well tolerated. The third injection, of 0.1 gm., at a similar interval did not produce much fall in temperature, but two animals died from peritonitis. The fourth, fifth and sixth injections were the same. After the conclusion of injections there were only three animals left. These and three controls were exposed to infection by eye with one drop of a suspension of *Br. abortus* equal to Gates No. 1 two weeks after the last injection. Another vaccinated animal died two weeks after exposure to infection. The spleen appeared normal and on culture was negative.

Agglutination tests were made weekly following exposure to infection and results are shown in Table III.

TABLE III

AGGLUTINATION TESTS ON GUINEA PIGS IN RESIDUE PROTECTION EXPERIMENT

		Infected						
		May 26	June 26	July 3	July 10	July 17	July 24	July 31
Vaccinated	2	—	1 : 25	1 : 25	1 : 25	Dead		
	4	—	1 : 100	1 : 100	1 : 100	1 : 100	1 : 250	1 : 500
	6	—	1 : 50	1 : 25	—	1 : 100	1 : 100	1 : 500
Controls	1			—	—	1 : 100	1 : 250	1 : 500
	2			—	—	1 : 100	1 : 250	1 : 500
	3			—	—	1 : 100	1 : 250	1 : 500

These animals were killed four weeks after exposure to infection. The spleens of all were slightly enlarged but no difference was observable between vaccinated animals and controls. *Br. abortus* was recovered from all. There was no evidence that any protection had developed from injections of this residue.

Intradermal test with residue. A suspension of 0.05 gm. of residue in 10 cc. of water was prepared. Half of this was treated by the addition of 0.25% formalin. Both were left in the refrigerator for two days. Normal

and infected guinea pigs were given intradermal injections of 0.1 cc. of formolized and untreated suspension. There was no reaction from either product in the negative animal but both produced a slight reaction in the infected guinea pig. It was not nearly as clear as that produced by the precipitate.

Experiment 3. Lot 3 supernatant residue. This was obtained from the supernatant fluids containing 2.5, 5 and 10 volumes of alcohol which were used to prepare precipitates in a previous experiment. It was prepared as in Experiment 2. Varying the amounts of alcohol did not appear to affect the residue. All three were about the same in complement-binding activity. The 5-volume precipitate and residue only need be given to illustrate the difference between the precipitate and residue. This is shown in Table IV.

TABLE IV

COMPLEMENT FIXATION TEST WITH 5-VOLUME PRODUCTS OF LOT 8

		1 : 25	1 : 50	1 : 100	1 : 250	1 : 500
Precipitate	1 : 25	Anticomplementary				
Residue	1 : 25	1	0	0	0	0
Precipitate	1 : 100	4	4	4	0	0
Residue	1 : 100	0	0	0	0	0
Precipitate	1 : 500	4	4	4	4	0
Residue	1 : 500	0	0	0	0	0

This residue gave only slight fixation in the first tube of the lowest dilution as compared with 1 : 250 in the 1 : 500 dilution of precipitate.

Guinea pigs were injected with 0.06 gm. of each residue. There was a rise of temperature in one animal and a very slight drop in the other two. All remained well. On the other hand three guinea pigs injected with 0.02 gm. of the precipitate at 2:00 p.m. were dead the following morning.

Evaporated Whole Filtrate

The alcoholic precipitate and its supernatant fluid residue having been tried, it was decided to obtain the solids in a filtrate with as little heating as possible. The bacteria were grown and handled by the usual method of producing alcoholic precipitate as far as the filtrate stage except that the suspension was not heated.

Experiment 4. Lot 1 evaporated whole filtrate (heated). Br. abortus was grown for five days on liver agar. It was washed off with salt solution, shaken for 24 hr., centrifuged and filtered through paper and a Mandler candle. The filtrate was evaporated down to about 20 cc. in the vacuum still. It was then dialyzed 48 hr. in running tap water, evaporated to dryness over a water bath between 70 and 80° C., and finished *in vacuo* over phosphorus pentoxide.

Intraperitoneal injection of guinea pigs. Two guinea pigs were given intraperitoneal injections of 0.02 gm. in 2.0 cc. water. Temperatures are shown in Table V.

TABLE V

INTRAPERITONEAL INJECTION OF EVAPORATED WHOLE FILTRATE

	9:15 a.m.	11:15 a.m.	2:00 p.m.	4:00 p.m.	6:00 p.m.	9:00 a.m.	11:45 a.m.	2:00 p.m.
No. 1	103.2	100.8	97.4	96.0	95.0	99.4	101.8	103.0
No. 2	103.6	101.0	96.8	97.2	97.2	Killed		

These animals showed the same symptoms as were produced by alcoholic precipitate. No. 1 recovered, No. 2 was killed as the rectum was everted. As would be expected, the temperatures did not go as low as with precipitate because less than 0.01 of the alcohol precipitable substance would be present in this injection.

Complement fixation test. The evaporated whole filtrate gave exactly the same results as the alcoholic precipitate prepared from the same filtrate. This was somewhat surprising in view of the difference in complement-binding value between precipitate and supernatant residue. The results from the evaporated whole filtrate are given in Table VI. The precipitate readings were identical.

TABLE VI

COMPLEMENT FIXATION TEST OF EVAPORATED WHOLE FILTRATE

	1 : 25	1 : 50	1 : 100	1 : 250	1 : 500	1 : 1000	1 : 2500
1 : 25	4	4	4	3	0	0	0
1 : 100	4	4	4	4	0	0	0
1 : 500	4	4	4	4	4	1	0

This substance showed the same tendency to inhibition in the lower dilutions that was manifested by the precipitate which was prepared from a portion of the same filtrate.

Experiment 5. Lot 1 evaporated whole filtrate (unheated). This lot differed from that in Experiment 4 in the fact that the suspension was not heated above 45° C. Forty-eight-hour growth of *Br. abortus* was washed off with water, shaken 22 hr. with glass beads, centrifuged and filtered through a fine candle. The clear filtrate was evaporated in the vacuum still with the water bath at about 45°C. It was not dialyzed. The last portion of the residual fluid was dried in the incubator at 37° C. and then over phosphorus pentoxide *in vacuo*. The finished product was very hygroscopic.

Intraperitoneal injection of evaporated filtrate. One guinea pig was given an intraperitoneal injection of 0.01 gm. in 2.0 cc. water and another was given 0.04 gm. Temperatures of these animals are shown in Table VII.

TABLE VII

INTRAPERITONEAL INJECTION OF EVAPORATED FILTRATE

	10:00 a.m.	12:00 m.	2:00 p.m.	4:00 p.m.	9:00 a.m.
No. 1 0.01 gm.	102.2	103.6	103.4	102.8	102.0
No. 2 0.04 gm.	102.8	98.0	95.0	94.0	Dead

The smaller injection produced a slight rise in temperature while the larger dose acted like alcoholic precipitate. It caused a rapid fall in temperature, and death.

Complement fixation test with evaporated filtrate. A suspension of 0.01 gm. in 2.0 cc. saline was diluted 1 : 25, 1 : 100 and 1 : 500.

These dilutions were set up in the ordinary way with a positive serum. The regular antigen was also set up as a check on this material. The results are shown in Table VIII.

TABLE VIII

COMPLEMENT FIXATION RESULTS WITH EVAPORATED WHOLE FILTRATE

		1 : 25	1 : 50	1 : 100	1 : 250	1 : 500	1 : 1000	1 : 2500
Lot 1 unheated	1 : 25	4	4	4	4	4	0	0
	1 : 100	0	0	0	0	0	0	0
	1 : 500	0	0	0	0	0	0	0
Regular antigen	1 : 15	4	4	4	4	4	4	0

The complement-binding power of this material was not nearly as great as that of the heated material in Experiment 4. This lot was only grown for two days as compared with five in the case of Experiment 4, which may be responsible for the difference.

Experiment 6. Lot 2 evaporated whole filtrate. Br. abortus was grown for three days on the usual liver agar. The growth was washed off with water and shaken for 24 hr. with glass beads. It was not heated. The suspension was centrifuged and filtered through a fine Mandler candle. A pane of glass was supported over a hot plate in such a manner that it merely became warm and not hot. A current of air was blown across the plate by means of a fan. The filtrate was poured on to cover the surface in a thin layer. It was dry in about two hours, when it was scraped off and the drying completed in a

desiccator under negative pressure over phosphorus pentoxide. It was ground up and tightly sealed in a vial. This material was very hygroscopic and became sticky during the short time required to weigh it. During the whole process it was not exposed to heat.

Complement fixation reaction. A suspension of 0.01 gm. in 2.0 cc. water was prepared. This was diluted to 1 : 25, 1 : 100 and 1 : 500 and set up with a positive serum and also the regular antigen. Results are given in Table IX.

TABLE IX

COMPLEMENT FIXATION TEST ON LOT 2 EVAPORATED WHOLE FILTRATE (UNHEATED)

		1 : 25	1 : 50	1 : 100	1 : 250	1 : 500	1 : 1000	1 : 2500	1 : 5000
Lot 2	1 : 25	4	4	4	4	4	4	4	0
	1 : 100	4	4	4	4	4	4	0	0
	1 : 500	4	0	0	0	0	0	0	0
Regular antigen	1 : 15	4	4	4	4	4	4	4	0

This lot of material differed from the first lot in that it did not show the tendency to inhibition in the lower dilutions and was not as strong an antigen. In these respects it resembled Lot 1 unheated (Exp. 5), but was active in a higher dilution than that product. Cultures from which the suspensions were prepared had been incubated one day longer.

Protection test with Lot 2 evaporated whole filtrate (unheated). Six guinea pigs were given intraperitoneal injections of this material, each receiving 0.01 gm. in 2 cc. water. No. 3 died after the third injection and showed the usual changes of inflammation of the peritoneum and fibrinous exudate on the liver. The others received six injections.

These animals were bled and exposed to infection with a suspension of *Br. abortus* equal to 1 cc. on the Gates nephelometer, two weeks after the last injection. There was no evidence of protection and *Br. abortus* was recovered from the spleens of these animals.

Precipitation of Filtrate with Ammonium Sulphate

Ammonium sulphate was tried in place of alcohol for precipitation of the filtrate of a suspension of *Br. abortus*. Only one experiment was carried out as it was considered unwise to change the methods of precipitating until the alcoholic precipitate had been more fully worked out.

Experiment 7. Ammonium sulphate precipitate. Br. abortus was grown for five days on liver agar. It was washed off with salt solution, heated, and shaken for 24 hr. It was then passed through a Sharples supercentrifuge and a fine Mandler candle. One portion of the clear filtrate was treated in the usual way with five volumes of 95% alcohol. To another lot of 200 cc. was

added 160 gm. of ammonium sulphate, C.P. Next morning the ammonium sulphate precipitate had collected on the surface of the fluid. It was removed, dialyzed, dried and ground. Guinea pig injections and complement fixation tests were carried out with both precipitates. Results of the complement fixation test are shown in Table X. The usual dilutions of antigen were employed.

TABLE X

COMPLEMENT FIXATION TEST WITH AMMONIUM SULPHATE AND ALCOHOL PRECIPITATES

		1 : 25	1 : 50	1 : 100	1 : 250	1 : 500	1 : 1000
Ammonium sulphate	1 : 25	4	4	4	4	0	0
Alcohol	1 : 25	4	4	4	3	0	0
Ammonium sulphate	1 : 100	4	4	4	4	2	0
Alcohol	1 : 100	4	4	4	4	0	0
Ammonium sulphate	1 : 500	4	4	4	4	0	0
Alcohol	1 : 500	4	4	4	4	4	1

The ammonium sulphate precipitate showed a little more fixation in the lower dilutions but in the 1 : 500 dilution of the suspension of these products (actually 1 : 100,000 dilution of the precipitates) the alcoholic precipitate was more antigenic.

Intraperitoneal injection of guinea pigs with both precipitates. Two guinea pigs injected with 0.02 gm. of the precipitates in 2.0 cc. water showed a similar fall in temperature and died the next day. Two more guinea pigs were injected with 0.02 gm. of alcoholic precipitate and two with the same dose of the ammonium sulphate product. The temperature drop was very similar in all four animals. One of the alcoholic-precipitate guinea pigs died and the other three animals recovered. It appeared from this that the material precipitated with ammonium sulphate was very similar to the alcoholic product.

Summary

The residue in the supernatant fluid after precipitation by alcohol was obtained by evaporation *in vacuo* at about 50° C., final drying at 70–80° C. for a short time over a water bath and then *in vacuo* over phosphorus pentoxide.

The residue was low in complement fixing power and was not as toxic for guinea pigs in equal dosage. Injections of 0.1 gm. proved fatal to these animals. Intradermal injections in infected guinea pigs produced only a slight reaction.

Two guinea pigs that had been given six injections of residue at five-day intervals and exposed to infection by eye two weeks after the last injection showed no evidence of protection. They were shown, by post-mortem and cultural examination, to be infected.

Filtrates of suspensions of heated and unheated *Br. abortus* were evaporated to dryness by vacuum distillation and low temperatures. The first lot prepared from a five-day growth was more efficient as a complement-fixing antigen than either of the other two lots, which had been grown for two and three days only, in spite of the fact that Lot 1 had been heated. The three-day growth product was stronger than that prepared from the two-day cultures. Intraperitoneal injections caused a fall in temperature in injected guinea pigs. Larger doses were required to kill than in the case of the precipitate. Intraperitoneal injections of this material did not protect guinea pigs against infection by eye. A precipitate produced with ammonium sulphate appeared to be the same as the alcoholic product as far as it was studied.

Acknowledgments

The writer gratefully acknowledges the assistance of T. Lloyd Jones, B.V.Sc., and the advice and support of Dr. H. B. Speakman and Dr. S. Hadwen in this work, which was made possible by a grant from the Department of Agriculture of Ontario.

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A STUDY OF VARIOUS FRACTIONS OF *BRUCELLA ABORTUS*

V. ALCOHOLIC PRECIPITATES PREPARED FROM A DISSOCIATED STRAIN OF *BR. ABORTUS* AND FROM *E. COLI* AND *B. SUBTILIS*[1]

By Ronald Gwatkin[2]

Abstract

An alcoholic precipitate from an R strain of *Brucella abortus* resembled the organism from which it was obtained. Toxicity was low, it produced only a slight reaction in the skin of an infected guinea pig and it had no antigenic power in the complement fixation test. An alcoholic precipitate of *E. coli* was more toxic than any obtained from *Br. abortus*. The effects of intraperitoneal injections of colon precipitate were modified by anti-colon serum. Intraperitoneal injection of an alcoholic precipitate of *B. subtilis* produced no change in guinea pigs other than a slight fall in temperature.

This article is a continuation of the work previously published on alcoholic precipitates and other portions of filtrates of *Br. abortus* (1–4). The experiments with *E. coli* and *B. subtilis* were introduced in order to see whether the toxicity found in the *Brucella* preparations would also occur in commensals and saprophytes. *E. coli*, which at times may be pathogenic, proved to be more toxic than any of the *Brucella* strains examined, but the toxicity of each was specific.

Experiment 1. Alcoholic precipitate of a dissociated strain of Br. abortus. An alcoholic precipitate was prepared from a dissociated strain of *Br. abortus*, the antigenic qualities of which have previously been reported (5, 6). The procedure was the same as that described for the regular strains (1). Centrifugation was simplified as the bacteria went out of suspension overnight.

Intraperitoneal injections. Three guinea pigs weighing about 350 gm. were given intraperitoneal injections of 0.01, 0.02 and 0.04 gm. of alcoholic precipitate. Temperatures of these animals are given in Table I.

TABLE I

Temperatures of guinea pigs injected with R alcoholic precipitate

	10:00 a.m.	12:00 m.	2:00 p.m.	5:00 p.m.	9:00 a.m.
No. 1 0.01 gm.	102.2	98.4	97.0	98.0	102.6
No. 2 0.02 gm.	102.0	101.2	100.0	98.2	102.4
No. 3 0.04 gm.	102.8	96.0	95.4	96.2	101.8

These animals showed symptoms the same as those produced by injections of S precipitate, but recovery was rapid and temperatures also returned quickly to normal in comparison with those of guinea pigs injected with the regular precipitate.

[1] *Manuscript received September 27, 1934.*
Contribution from the Department of Pathology and Bacteriology, Ontario Research Foundation, Toronto, Canada.
[2] *Research Fellow.*

Intradermal test with R alcoholic precipitate. A normal and an infected guinea pig were injected intradermally with 0.1 cc. of a 1 : 250 suspension of precipitate. There was no reaction in either animal at 24 hr. but the following day there was a slight reaction in the infected guinea pig, which was not equal to that produced by the regular precipitate.

Complement fixation test. A suspension of 0.01 gm. of R precipitate in 2 cc. saline was prepared. This was diluted 1 : 25, 1 : 100 and 1 : 500, making dilutions of the precipitate of 1 : 5000, 1 : 20,000 and 1 : 100,000. These were the dilutions used for the regular precipitate. Results are shown in Table II.

TABLE II

COMPLEMENT FIXATION TEST WITH R ALCOHOLIC PRECIPITATE

		1 : 25	1 : 50	1 : 100	1 : 250	1 : 500	1 : 1000	1 : 2500
	1 : 25	0	0	0	0	0	0	0
	1 : 100	0	0	0	0	0	0	0
	1 : 500	0	0	0	0	0	0	0
Lot 6	1 : 500	4	4	4	4	2	0	0
Reg. antigen	1 : 15	4	4	4	4	4	4	0

A positive serum with complement binding titre of 1 : 1000 did not produce any fixation with R precipitate in the same dilutions in which S precipitate and the regular antigen showed definite fixation of complement.

The results from R alcoholic precipitate coincide with the behavior of suspensions of the R organism. In the work to which reference has been made, R had no antigenic value in the complement fixation test. It was harmless for guinea pigs until after a series of animal passages but it did produce a skin reaction in infected guinea pigs. No further work was carried on with R precipitate. Earlier work with this organism itself had shown that living cultures had no immunizing value against subsequent exposure to infection with the S prototype.

Experiment 2. Alcoholic precipitate of E. coli. An alcoholic precipitate was prepared from a strain of colon isolated from feces. The preparation was as already described. The precipitate appeared to be more soluble than that prepared from *Br. abortus* and there was no difficulty in getting it into solution.

Intraperitoneal injection of guinea pigs with colon precipitate. Two guinea pigs were given intraperitoneal injections of colon precipitate. No. 1 received 0.01 gm. and No. 2 was given 0.04 gm. in 2 cc. water. Death occurred as rapidly, and, in the larger dose, more rapidly than following *Brucella* precipitate. Temperatures are shown in Table III. Symptoms were the same as those produced by *Brucella* precipitate.

TABLE III

TEMPERATURES OF GUINEA PIGS INJECTED WITH COLON PRECIPITATE

	10:00 a.m.	12:15 p.m.	2:00 p.m.	4:00 p.m.	9:00 a.m.
No. 1	102.6	94.0	94.0	94.0	Dead
No. 2	102.8	94.0	94.0	Dead	—

It will be seen that the temperature of the injected animals dropped very rapidly, even in the case of the smaller dose, and death of the animal receiving the larger dose occurred five hours after injection.

Effect of anti-abortus serum on injections of colon precipitate. In view of the toxicity of this substance and to make sure that the action of alcoholic precipitate was not merely a general one, common to all organisms and without specificity, guinea pigs were given intraperitoneal injections of this material and normal and immune serum. The latter was rabbit serum which had proved so effective in a previous experiment (3). Temperatures of these animals are shown in Table IV. No. 1 received 5.0 cc. immune serum and 0.02 gm. of precipitate while No. 2 was given 5.0 cc. negative serum and 0.02 gm. precipitate.

TABLE IV

TEMPERATURES OF GUINEA PIGS INJECTED WITH NORMAL AND ANTI-ABORTUS SERUM AND COLON ALCOHOLIC PRECIPITATE

	11:00 a.m.	12:30 p.m.	2:00 p.m.	3:30 p.m.	5:00 p.m.	9:00 a.m.
No. 1	102.8	94.0	94.0	94.0	94.0	Dead
No. 2	102.8	97.0	94.0	94.0	94.0	Dead

In 1½ hours the temperature of the animal that received anti-abortus serum and precipitate had dropped to 94. On the next reading the normal-serum animal was also 94. Both were dead the following morning. No anti-colon serum was available at that time to try the effect of a specific anti-serum on colon precipitate, but the experiment showed that anti-abortus serum capable of protecting guinea pigs against *Brucella* precipitate had no effect against colon precipitate.

Experiment 3. Effect of anti-colon serum on colon alcoholic precipitate. A rabbit was given three injections of phenolized suspension of *E. coli* by the subcutaneous route, followed by an injection of living culture. Three weeks after the last injection, blood was drawn from the heart, the serum was collected and was heated at 56° C. for 30 min. An alcoholic precipitate had been prepared from the same strain. The serum had an agglutinin titre of only 1 : 100. Blood was collected from a normal rabbit, and when this was

examined later for agglutinins against *E. coli* it was found that partial agglutination occurred in 1 : 10, 1 : 25 and 1 : 50, which would account for the protection afforded by this supposedly negative serum. Three guinea pigs were injected by the intraperitoneal route as follows:

1. 0.01 gm. alcoholic ppt. + 6 cc. water
2. 0.01 gm. alcoholic ppt. + 6 cc. normal (?)serum.
3. 0.01 gm. alcoholic ppt. + 6 cc. anti-colon serum.

Temperatures of these guinea pigs are shown in Table V.

TABLE V

TEMPERATURES OF GUINEA PIGS

	10:00 a.m.	12:00 m.	2:00 p.m.	4:00 p.m.	5:00 p.m.	9:00 a.m.	11:00 a.m.	12:00 m.	9:00 a.m.
No. 1	102.0	93.0	93.0	93.0	93.0	93.0	93.0	93.0	Dead
No. 2	101.2	97.0	97.0	97.4	98.0	98.8	100.0	101.0	102.0
No. 3	101.4	100.2	100.8	97.2	96.0	99.0	101.8	102.0	102.2

The temperature of the guinea pig that received precipitate alone dropped very rapidly and the animal died. The temperature of the anti-colon-serum guinea pig came down more slowly and went up more rapidly than that of the animal which received the serum from an uninjected rabbit. This serum, however, contained agglutinins against *E. coli*, and both serum-treated rabbits recovered.

Experiment 4. Alcoholic precipitate of B. subtilis. This organism was grown on liver agar and an alcoholic precipitate was prepared. *B. subtilis* was chosen as being one which could not be considered to be pathogenic at any time, whereas the writer has had many virulent strains of *E. coli* from animals. The precipitate was similar in appearance to *Brucella* and colon preparations. Two guinea pigs were given intraperitoneal injections of the precipitate. No. 1 received 0.01 gm. in 2.0 cc. water and No. 2 was given 0.04 gm. in 2 cc. water. Temperatures of these animals are given in Table VI.

TABLE VI

TEMPERATURES OF GUINEA PIGS INJECTED WITH *B. subtilis* PRECIPITATE

	9:30 a.m.	11:15 a.m.	2:30 p.m.	4:00 p.m.	9:15 a.m.	11:00 a.m.	2:30 p.m.	4:00 p.m.	9:00 a.m.
No. 1	103.0	103.0	101.0	99.4	102.6	101.6	102.0	100.6	102.2
No. 2	103.1	101.2	102.8	102.6	100.0	101.2	101.2	100.4	101.4

There was some drop in temperature in these guinea pigs but they appeared well throughout the period of observation, and there was not the illness associated with *Brucella* and colon precipitate.

Summary

Intraperitoneal injections of R alcoholic precipitate produced symptoms similar to those following injections of S precipitate. They were less marked and the temperature returned more rapidly to normal. A slight skin reaction was produced by R precipitate in an infected guinea pig. This was not as well marked as the S-precipitate reaction although the suspension of R was stronger. The R precipitate did not react as an antigen in the complement fixation test.

An alcoholic precipitate of *E. coli* produced a more violent reaction in guinea pigs than the *Brucella* precipitate. The effect of intraperitoneal injections of colon precipitate was not modified by anti-abortus serum. Guinea pigs were protected by serums containing colon antibodies.

An alcoholic precipitate of *B. subtilis* produced some fall in temperature following the intraperitoneal injection, but no other disturbance was noticed in the injected guinea pigs.

Acknowledgments

The writer is grateful to T. Lloyd Jones, B.V.Sc., for his assistance and to Dr. H. B. Speakman and Dr. S. Hadwen for advice and support in connection with this work, which was made possible by a grant from the Department of Agriculture of Ontario.

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A STUDY OF VARIOUS FRACTIONS OF *BRUCELLA ABORTUS*

VI. EXTRACT OF DRIED AND GROUND ORGANISMS[1]

By Ronald Gwatkin[2]

Abstract

Filtrates prepared from dry, ground *Brucella abortus* (Huddleson) caused a fall in temperature and death in some cases in guinea pigs injected by the intraperitoneal route. The filtrates were not as active as bacterial suspension antigen in the complement fixation test. They produced reactions in the skin of infected guinea pigs. Intraperitoneal injections failed to protect guinea pigs against infection by eye with *Br. abortus*. The preparation of this material gave rise to marked symptoms in a hypersensitive human subject.

Introduction

In previous papers the writer has recorded experiments with alcoholic precipitate and various residue antigens (1–5). This article deals with a filtered extract of dried and ground *Br. abortus*.

This method was explained to the writer by Dr. I. Forest Huddleson of Michigan. Dr. Huddleson found that this extract was toxic for guinea pigs but it did not apparently have any immunizing value.

Flat bottles of liver agar, pH 6.6, were seeded with an aerobic culture of *Br. abortus* of recent isolation. After three days at 37° C., the growth was washed off with saline, centrifuged, and the sediment dried for a day in the incubator and then over phosphorus pentoxide *in vacuo*. The dried material was ground for 24 hr. in a ball mill with $\frac{3}{8}$-in. steel balls. Salt solution containing 1% toluene and 1% ether was then added and the mixture was rotated for a couple of hours. It was then incubated at 37° C. for 48 hr. The next step was filtration through paper treated with fuller's earth, after which it was heated for 10 min. at 100° C. and concentrated by evaporation with a fan and a current of warm air. It was then filtered through a fine Mandler candle.

Guinea pig inoculation. One guinea pig was given an intraperitoneal injection of 5.0 cc., while a second received 1.0 cc. by the same route. Both animals twitched for a few minutes after injection and then settled down quietly. The first animal became very ill within a few hours and its temperature dropped from 102.8 to 98.0° F. The following day it was 94.0 (the lowest our thermometer would record) and it remained there until the following day when it rose to 101.4. The temperature of the other guinea pig did not go below 97.6. Both recovered.

[1] *Manuscript received September 27, 1934.*
Contribution from the Department of Pathology and Bacteriology, Ontario Research Foundation, Toronto, Canada.
[2] *Research Fellow.*

Four more guinea pigs were injected with doses of from 5.0 cc. to 0.01 cc. by the intra-abdominal route at 2:30 p.m. Table I gives injections and temperature reactions of these animals.

TABLE I

GUINEA PIGS INJECTED WITH GROUND EXTRACT FILTRATE

No.	Injection, cc.	Temperatures			
		2:30 p.m.	3:30 p.m.	5:30 p.m.	9:00 a.m.
1	5.0	102.8	97.2	94.0	Dead
2	1.0	102.4	101.0	101.2	101.8
3	0.1	102.4	104.2	103.0	103.0
4	0.01	102.6	102.4	103.4	101.8

No. 1 died during the night. There was fluid in the abdominal cavity, fibrinous exudate on liver, inflammation of peritoneum and intestines. Cultures were negative.

In order to determine what effect might be produced by the medium itself, 5.0 cc. of liver agar was melted and 10.0 cc. saline added to keep it liquid. An injection of 5.0 cc. of this produced a drop in temperature of only 1.5°F.

The five surviving guinea pigs from the above series and three normal guinea pigs were exposed to infection by eye with one drop of a suspension of *Br. abortus* equal in density to tube No. 2 of McFarland's nephelometer, three weeks after they had received the injections of filtrate. Agglutination tests were carried out weekly and the animals were killed 42 days after exposure to infection. There was no suggestion of any protection in the injected animals, all being shown to be infected.

Ground extract filtrate II. Another filtrate was prepared in the same manner as the first one, except that chloroform in excess was added during the period of extraction in place of toluene, and the filtrate was not concentrated by evaporation. Two guinea pigs injected with 5.0 cc. and 1.0 cc. showed a drop in temperature but survived.

Ground extract filtrate III. A third filtrate produced death in a guinea pig injected with 5.0 cc. The animal lived two days and there was a large amount of sterile pus in the abdominal cavity. This filtrate consisted of 10 gm. of dry powder in 250 cc. saline and was produced in the same manner as filtrate No. 1.

Complement fixation tests with Lots 1, 2 and 3. Filtrate No. 1 was diluted 1 : 25 and 1 : 1000. Lots 2 and 3 were diluted 1 : 10 and 1 : 100 and all were used as antigens in the complement fixation test with a positive serum. The tests were carefully controlled with antigen and serum tubes, details of which need not be shown here. Table II gives the results with these and our regular *Br. abortus* antigen.

TABLE II

COMPLEMENT FIXATION TEST ON LOTS 1, 2 AND 3

		1 : 25	1 : 50	1 : 100	1 : 250	1 : 500	1 : 1000	1 : 2500
Lot 1	1 : 25	4	4	0	0	0	0	0
	1 : 1000	4	4	4	2	0	0	0
Lot 2	1 : 10	4	4	1	0	0	0	0
	1 : 100	4	4	4	0	0	0	0
Lot 3	1 : 10	4	4	4	2	0	0	0
	1 : 100	4	4	4	0	0	0	0
Regular antigen		4	4	4	4	4	4	0

4 = Complete fixation. 3 = 75% fixation. 2 = 50% fixation. 1 = 25% fixation. 0 = No fixation.

It will be noted that the filtrates were not as strong antigens as the regular suspension of *Br. abortus.* This was a bacterial suspension of the organism of the same density as the agglutination antigen (No. 1, McFarland's nephelometer) diluted 1 : 15 for use in the complement fixation test. In Lot 1 the 1 : 1000 dilution showed more fixation than 1 : 25. The same was seen with Lot 2 in dilutions of 1 : 10 and 1 : 100. Lot 1 was repeated with the same result in 1 : 25 and a 1+ instead of a 2+ in the last tube of the 1 : 1000 dilution, which is within the range of experimental error. The same inhibition had been observed with the various alcoholic precipitates.

Skin tests. Infected and normal guinea pigs were given intradermal injections of 0.1 cc. of two of the filtrates. Lot 2 gave some reaction in both infected and normal animals but that in the latter disappeared after two days, while a small, hard swelling persisted in the infected one. Lot 3 gave a well marked swelling in the infected pig but produced no reaction in the normal animal. The preparation of this material produced the same symptoms in a hypersusceptible human subject as were previously recorded for the alcoholic precipitate (1).

Immunity experiment. Four guinea pigs were given six intraperitoneal injections of filtrate No. 3 over a period of two weeks. The first five injections were 2.0 cc. each and the sixth was 4.0 cc. One week after the last injection these animals and two normal guinea pigs were exposed to infection by eye with one drop of *Br. abortus* suspension equal in density to tube No. 2 of McFarland's nephelometer. The only pregnant female in the vaccinated group aborted 17 days after infection. These animals were killed a month after exposure, weekly agglutination tests having shown that all were infected. Post-mortem examination showed enlargement of the spleen. Cultures were positive. There was no delay in agglutinin production in the vaccinated pigs, nor any other indication that the injections of filtrate had done anything to delay the onset of infection.

Summary

A filtrate was prepared from dry, ground *Br. abortus* as suggested by Dr. I. Forest Huddleson. Intraperitoneal injections caused a fall of temperature in guinea pigs and, in some cases, death. The filtrates were not as strong complement-binding antigens as a bacterial suspension. There was a tendency to inhibition of fixing power in the lower dilutions. The filtrates produced skin reactions when injected into infected guinea pigs and, in one case, in a normal animal also. Six injections of filtrate over a period of two weeks failed to protect guinea pigs against subsequent infection by eye.

The preparation of this material gave rise to symptoms simulating undulant fever in a hypersensitive human subject.

Acknowledgments

The writer is grateful to Dr. I. Forest Huddleson for his interest in the problem and to Dr. H. B. Speakman and Dr. S. Hadwen for advice and support throughout this work, which was made possible by a grant from the Department of Agriculture of Ontario.

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SOME OBSERVATIONS ON THE ALLERGY TO TUBERCULIN OF GUINEA PIGS VACCINATED WITH B.C.G.[1]

By Armand Frappier[2] and Victorien Fredette[3]

Abstract

Experiments indicate that the development of allergy in guinea pigs vaccinated with B.C.G. (0.0025, 0.005 or 0.010 gm.), is relatively slow and varies with individuals. It is possible to measure the degree of skin sensitivity by serial intradermal tests with decreasing doses of tuberculin. Classification as to degree of allergy and study of the development of hypersensitivity can hardly be effected on the basis of strong doses of tuberculin. In fact, early positive reaction is not always a sign of lasting hypersensitivity and many guinea pigs, which respond at the same time to a strong dose of tuberculin, can be differentiated as to degree of hypersensitivity by tests with smaller doses of tuberculin. Most of the subcutaneously vaccinated guinea pigs developed allergy in from five to twenty days. The maximum allergy was reached in from one to two months. In a large number of the more sensitive animals the dose was then reduced to 0.00001 cc. and the reaction was maintained during the third and fourth months and perhaps longer. A few animals were sensitive to 0.000005 cc. in the fourth month.

The observation has been verified that in subcutaneously vaccinated guinea pigs there is, as a rule, no correlation between small variations in the size of the dose of B.C.G. and the degree of allergy produced. Nevertheless, of the animals vaccinated intraperitoneally, those inoculated with the larger quantities of bacilli were more highly sensitized. This distinction was made by means of the smaller doses of tuberculin.

Introduction

Most authors appear to agree as to the innocuousness of B.C.G. They apparently consider the attenuation of this virus to be constant and irreversible. They also admit that B.C.G., introduced into the organism, gives rise to a certain "premunition" against virulent tuberculous infections, and a more or less pronounced allergic or hypersensitive state. But a battle wages around the quantitative appreciation of the value and length of the immunity and hypersensitivity thus conferred.

There is no doubt that the problems of tuberculous infection in general will be more easily solved with the advent of more precise understanding of the effects of immunity and hypersensitivity on its evolution. This is the actual reason why most workers turn to the exploration of compared limits of allergy and immunity in tuberculosis.

Following Romer (24), Hamburger (16), Debré *et. al.* (14, 15), Paraf (19), Bezancon and de Serbonnes (1), and others, many authors, *e.g.*, Boquet *et. al.* (7–13), Saenz (28, 29), Birkhaug (2), and Petroff (21–23), have undertaken to study the ante-allergic and allergic periods in animals inoculated by different modes, with different doses of dead or living tubercle bacilli of varied virulence, or extracts of tubercle bacilli. The test dose was generally of strong concentration.

[1] *Original manuscript received July 21, 1934. Contribution from the B.C.G. Laboratories, Department of Bacteriology, Faculty of Medicine, University of Montreal, Montreal, Quebec, Canada.*

[2] *Director of the Departments of Bacteriology, University of Montreal, and St. Luke's Hospital, Montreal.*

[3] *Bacteriologist, University of Montreal.*

Although the earliest and more lasting reactions to strong doses of tuberculin are often characteristic of the most sensitive animals, these cannot serve either to measure effectively the degree of hypersensitivity or to follow its progressive development in any animal.

Long ago Koch (17, 18) noted that, in infected animals, the lethal dose of tuberculin decreases with the elapsed time since infection. Borrel (3), confirmed this finding. In 1929 Parish and Okell (20) reported their observations on the development of hypersensitivity to intradermal doses of tuberculin. They found that in guinea pigs infected with a large quantity of virulent bacilli (0.5 mg.) the doses decreased with time from 0.004 to 0.00005 cc.

The work thus begun appears to have been neglected by the majority of workers who failed to measure the degree and development of allergy by means of serial and intradermal reactions to decreasing doses of tuberculin. Should the results obtained with this procedure prove to be reliable it would be interesting to study the developments of immunity in the light of the allergy behavior thus determined.

The work of determining, by serial and intradermal reactions effected with decreasing doses of tuberculin, the development of hypersensitivity in our guinea pigs inoculated with B.C.G., has been undertaken. This preliminary research will serve in a proposed study of the relations between the states of allergy and immunity in these vaccinated animals.

This work was already under way when Boquet and Bretey (4, 5) published interesting results along this line. Their first notes dealt with guinea pigs infected by virulent, semi-virulent or dead bacilli. In their latest paper (6), a small part of which treats of B.C.G., they have published graphs which show that, for doses of 0.001 or 0.0001 cc. of B.C.G., inoculated subcutaneously, intradermally or intraperitoneally, the skin sensitivity begins to manifest itself by a positive reaction to 0.01 cc. of tuberculin, about the eighteenth day after inoculation. It increases little by little until the end of the third or fourth month, at which time the testing dose has been lowered to 0.0001 cc. after which the sensitivity diminishes by steps.

We were interested in having more details about B.C.G. infected guinea pigs. Our animals are divided into groups injected with doses of B.C.G. stronger than those used by the above authors, the doses varying relatively little from one group to another, in order to study the correlations between small differences in the quantity of bacilli injected and the degree of allergy produced. It was thought worth while to show what happens among all the animals and this is why tables and graphs are presented to show the percentages of animals reacting to the different doses of tuberculin at successive periods after vaccination. The graphs relating to the most sensitive guinea pigs will be produced later. For the moment, we are not in position to say more than that our results are not in conflict with those of Boquet and Bretey.

The B.C.G. weekly emulsion, used for vaccinating new-born babies at the University of Montreal, is always prepared under the same rigorous conditions, and its composition may be regarded as constant. By means of guinea pig controls of these weekly emulsions of B.C.G. (*i.e.*, animals inoculated with the respective doses of 0.010, 0.005 and 0.0025 gm. of B.C.G.), three groups of about the same weight (400 gm.), the same age and of pale color, have gradually been formed. Before vaccination, these have been tested twice intradermally, with a one-week interval, with a dose of 0.1 cc. of a $\frac{1}{10}$ dilution of tuberculin from the Institut Pasteur. Tuberculin of the same lot and of constant activity was always used. The dilutions prepared the same day as the tests were made were calculated so that a 0.1-cc. dose might be administered. The syringe used was Dr. Kuss', made by Gentile Co. of Paris, and capable of delivering, by means of its cursor piston, $\frac{1}{80}$ cc. The animals' sides were epilated on surfaces 3 cm. in diameter.

After B.C.G. inoculation, the guinea pigs were tested about every five days at different points of their skin with a similar dose of tuberculin (*i.e.*, 0.01 cc.), until a positive response was obtained. These animals then received three doses of diluted tuberculin, *viz.* 0.001 cc., 0.0001 cc., and 0.00001 cc. at the same time but at different points of their skin. If any animals still reacted with the last dose, they were inoculated immediately with two smaller doses, namely 0.000005 and 0.0000025 cc. and so on, dividing the previous dose by two until the limit dose still active was obtained.

During the first two months, the tests were made about every week; during the third month, every two weeks; and from then on, once a month, by repeating the last positive dose and the next smaller one. For any dose of tuberculin, the positive reactions which turned negative were controlled by two subsequent tests at a week's interval.

The results were read at 24, 48 and 72 hours, and the positive reactions expressed as follows:

+ = well defined swelling of the skin of about 0.5 cm. in diameter.
++ = the same, but of 0.7 cm. in diameter.
+++ = the same, but of 1.0 cm. in diameter.
++++ = the same, but of 1.5 cm. in diameter.
+++++ = the same, but of 2 cm. and more in diameter.

When observed, the hemorrhagic or necrotic lesions were noted.

Similar experiments were carried out on a control group of at least ten non-vaccinated guinea pigs. The fact that we have never noted any positive or suspect reactions among this group assures us of the specificity of the reactions obtained among vaccinated animals.

Consecutive doses of tuberculin were never injected twice in the same area of the skin, when it was possible to avoid it, in order to eliminate the activation of homeotropic reactions, as described by von Pirquet (30) and

Weiss (31) for humans, although Boquet and Valtis (13) have demonstrated that, contrary to the opinion of Romer and Joseph (25, 26, 27), such an activation does not take place in guinea pigs as it may do in humans, since the guinea pig's skin is less sensitive to tuberculin than human skin.

To check this contention of Boquet and Valtis, their tests have been carried out on about 20 guinea pigs already in allergic state and on a few control animals. Varying doses of tuberculin were injected upon the seat of former reactions, which had given positive or negative results about seven days before, and at the same time on the same animals, similar doses were inoculated as a control in a new area of the skin. No activation or weakening of the reactions has been observed, either among the vaccinated or among the control group. Moreover it is generally admitted that tuberculin does not sensitize by itself.

Variations in weather do not seem to affect the reactivity of the skin. Even after brisk changes of temperature, atmospheric pressure and humidity, such as occurred during the past winter, our guinea pigs proved to be as sensitive as in hot weather. Nevertheless, we avoided making tests during very cold and moist weather.

Among twenty pregnant animals, a loss of skin reactivity a few weeks before and after delivery has been observed. The same phenomenon occurred during intercurrent diseases such as broncho-pneumonia, etc., that usually kill the guinea pigs. All these animals were discarded from the experiment and do not figure in the results. More than 2000 tuberculin tests have been made upon the 54 guinea pigs under experiment.

Table I shows how the weekly succession of vaccinations and the selection of subjects cause variations in the number of animals under experiment for every month after inoculation. Thus in July, at the beginning of our work, it was possible for us to test only two subjects in the group of guinea pigs subcutaneously vaccinated with 0.005 gm. of B.C.G. One of these was vaccinated a month before and the other two months before. In August, these animals, therefore, passed into the third and fourth months after vaccination. On the other hand, two newly vaccinated guinea pigs were examined in their first month of vaccination. The sum of the guinea pigs observed during the course of each monthly period following vaccination represents the total number on which our results for each corresponding period are based.

TABLE I

EXAMPLE OF THE PROGRESSIVE COMPOSITION OF THE MONTHLY GROUPS

—	Months after vaccination				
	1	2	3	4	5
July	0	1	1	0	0
August	2	0	1	1	0
September	3	2	0	1	1
October	2	3	2	0	1
November	4	2	3	2	0
December	0	4	2	3	2
Totals	11	12	9	7	4

It sometimes happens that a total monthly number is smaller than that of the preceding month since some animals, already negative to tuberculin, do not figure at this period. In this special case, the percentage of positive reactions was then calculated on the number of animals of the preceding month. In this way, these percentages are, if anything, lower.

Results

Allergy among Subcutaneously Vaccinated Guinea Pigs

Table II gives in round numbers the percentages of animals, vaccinated subcutaneously with the three doses of B.C.G., which have reacted to decreasing doses of tuberculin with an intensity of at least +. Data obtained so far cover a period of four months.

TABLE II

PERCENTAGES OF GUINEA PIGS, SUBCUTANEOUSLY VACCINATED WITH B.C.G., WHICH ACQUIRED HYPERSENSITIVITY TO DECREASING DOSES OF TUBERCULIN AT DIFFERENT PERIODS AFTER VACCINATION

Doses B.C.G., gm.		0.01 cc. tuberculin									0.0001 cc. tuberculin								
	Days	5	10	15	20	25	30	60	90	120	5	10	15	20	25	30	60	90	120
A	No. animals used	5	6	7	8	8	8	9	8	6	5	6	7	8	8	8	9	8	6
0.010	No. giving positive response	1	2	5	7	8	8	9	7	5	0	0	0	1	2	3	6	4	3
	Reactors, %	20	33	71	87	100	100	100	87	83	0	0	0	13	25	37	66	50	50
B	No. animals used	7	7	10	10	11	11	12	9	7	7	7	10	10	11	11	12	9	7
0.005	No. giving positive response	0	0	6	7	9	9	12	8	6	0	0	1	2	4	5	6	4	3
	Reactors, %	0	0	60	70	81	81	100	88	84	0	0	10	20	36	45	50	45	42
C	No. animals used	4	4	4	6	6	6	8	5	5	4	4	4	6	6	6	8	5	5
0.0025	No. giving positive response	0	2	3	5	5	5	8	4	5	0	0	0	2	3	3	4	3	3
	Reactors, %	0	50	75	83	83	83	100	80	100	0	0	0	33	50	50	50	60	60
		0.001 cc. tuberculin									0.0001 cc. tuberculin								
A	No. animals used	5	6	7	8	8	8	9	8	6	5	6	7	8	8	8	9	8	6
0.010	No. giving positive response	0	0	2	5	7	7	7	6	3	0	0	0	0	0	0	2	3	1
	Reactors, %	0	0	28	62	87	87	77	75	50	0	0	0	0	0	0	22	37	17
B	No. animals used	7	7	10	10	11	11	12	9	7	7	7	10	10	11	11	12	9	7
0.005	No. giving positive response	0	0	4	5	9	9	8	7	6	0	0	0	0	0	0	0	0	1
	Reactors, %	0	0	40	50	81	81	75	77	84	0	0	0	0	0	0	0	0	14
C	No. animals used	4	4	4	6	6	6	8	5	5	4	4	4	6	6	6	8	5	5
0.0025	No. giving positive response	0	0	2	3	5	5	7	4	4	0	0	0	0	1	2	4	3	2
	Reactors, %	0	0	50	50	83	83	87	80	80	0	0	0	0	17	33	50	60	40

Allergy Among Intraperitoneally Vaccinated Guinea Pigs

At the beginning of our experiments, a series of intraperitoneal inoculations in guinea pigs had already been completed and there were at hand a great number of these animals vaccinated with the three doses of B.C.G. mentioned above, the last vaccination having occurred four months previously. They had been tested with tuberculin previous to their vaccination and found negative.

It was thought to be of interest to experiment on them with the same method used for the subcutaneously vaccinated animals, despite the length of time since their vaccination, and the results of observation of this incomplete series are given here. The observation extended from the fourth to the tenth month after vaccination (Table III).

Another series of intraperitoneal vaccinations has recently been begun and data which will give an idea of the progression of hypersensitivity to decreasing doses of tuberculin during the first four months after intraperitoneal vaccination will soon be available.

Table III gives the numbers and percentages of animals vaccinated with the three doses of B.C.G. mentioned above, which reacted positively to decreasing doses of tuberculin with an intensity of at least +.

TABLE III

PERCENTAGES OF GUINEA PIGS, INTRAPERITONEALLY VACCINATED WITH B.C.G., WHICH HAVE ACQUIRED HYPERSENSITIVITY TO DECREASING DOSES OF TUBERCULIN IN PERIODS OF FOUR TO TEN MONTHS AFTER VACCINATION

Doses B.C.G., gm.		0.010 cc. tuberculin							0.0001 cc. tuberculin						
	Months	4	5	6	7	8	9	10	4	5	6	7	8	9	10
A 0.010	No. animals used	4	5	7	7	8	7	6	4	5	7	7	8	7	6
	No. giving positive response	4	5	7	7	8	6	5	4	5	7	5	6	6	5
	Reactors, %	100	100	100	100	100	86	83	100	100	100	71	75	86	83
B 0.005	No. animals used	4	6	7	6	6	6	6	4	6	7	6	6	6	6
	No. giving positive response	4	6	7	6	5	6	5	2	2	5	3	3	3	3
	Reactors, %	100	100	100	100	83	100	83	50	33	71	50	50	50	50
C 0.0025	No. animals used	5	6	9	10	9	9	7	5	6	9	10	9	9	7
	No. giving positive response	5	6	8	10	9	8	6	5	4	7	6	5	5	3
	Reactors, %	100	100	88	100	100	88	86	100	67	77	60	55	55	43
		0.001 cc. tuberculin							0.00001 cc. tuberculin						
A 0.010	No. animals used	4	5	7	7	8	7	6	4	5	7	7	8	7	6
	No. giving positive response	4	5	7	6	7	6	5	2	4	3	3	4	3	4
	Reactors, %	100	100	100	86	88	86	83	50	80	43	43	50	43	67
B 0.005	No. animals used	4	6	7	6	6	6	6	4	6	7	6	6	6	6
	No. giving positive response	4	5	6	5	3	5	4	0	2	4	1	1	2	0
	Reactors, %	100	83	86	83	50	83	67	0	33	57	17	17	33	0
C 0.0025	No. animals used	5	6	9	10	9	9	7	5	6	9	10	9	9	7
	No. giving positive response	5	6	8	8	6	8	4	1	2	3	2	4	4	3
	Reactors, %	100	100	88	80	67	88	57	20	33	33	20	44	44	42

Comments

A few facts observed may be pointed out with all the reserve necessary for unfinished work.

(*i*). Well-marked differences are noted from one guinea pig to another in the establishment and regression of the sensitivity to decreasing doses of tuberculin.

Theoretically the development of allergy in the most sensitive animal is characterized by the earliest reactions to smaller and smaller doses of tuberculin, which reactions are maintained for the longest period of time. In practice several guinea pigs which react quickly to small doses of tuberculin do not maintain these reactions as long as other guinea pigs of less quickly developed but more lasting sensitivity.

Fig. 6, which takes account as much as possible of the above theoretical definition, illustrates the individual curves of the most sensitive guinea pigs vaccinated subcutaneously. This is discussed under (*vii*). However, the following information shows that the actual classification of each individual as to its degree of allergy, may be effected at any time by testing the animals with decreasing doses of tuberculin.

(*ii*). The tables do not show the results obtained with doses of tuberculin smaller than 0.00001 cc. Among the guinea pigs subcutaneously vaccinated with the larger doses of B.C.G., the positive reactions to the minimum dose of 0.000005 cc. of tuberculin appeared only on two; on the 99th day for the animal which received 0.010 gm. of B.C.G. and on the 125th day for the one vaccinated with 0.005 gm. (Figs. 5 and 6).

On three animals vaccinated intraperitoneally with the quantities of B.C.G. mentioned above, very definite reactions for 0.000005, 0.0000025 and even 0.00000166 cc. of tuberculin have been sustained. These reactions were observed at about the sixth month and persisted at the tenth month.

(*iii*). In the light of the facts exposed, it is justifiable to assume that, when a positive reaction is obtained with small doses of tuberculin, a larger dose would also have produced a positive reaction, on the condition that the tuberculin be administered at the same time on the same animal and, as far as possible, at comparable points of the skin.

(*iv*). At any period after vaccination, the percentage of positive animals diminishes, in general, relative to the concentration of the dose of tuberculin. Thus at about the second month after vaccination, the percentage of positive animals vaccinated subcutaneously with 0.010 gm. of B.C.G., is about at its peak and is divided as follows:

100% for 0.01 cc. of tuberculin.
77% for 0.001 cc. of tuberculin.
66% for 0.0001 cc. of tuberculin.
22% for 0.00001 cc. of tuberculin.

The animals may then be classified at any period according to their degree of allergy, by their response to decreasing doses of tuberculin, the animals reacting to 0.00001 cc. being considered the most sensitive.

(*v*). For a given dose of B.C.G. administered subcutaneously, the length of the ante-allergic period increases inversely as the concentration of the dose of tuberculin. The delay is greatest for the smallest doses.

(*vi*). The intensity of the reactions to tuberculin of the B.C.G.-inoculated guinea pigs varies certainly with the dose of tuberculin itself and with the length of time, but there is also a great variation in the intensity of the reaction which must be ascribed to the individual factor. The positivity of the reaction, however, is not affected by the individual factor to the same extent as the intensity.

For a given very sensitive animal, if the reaction to 0.01 cc. of tuberculin manifests itself with an intensity of 4+ or 5+, the reaction to 0.001 cc. will generally be 3+ or 4+; to 0.0001 cc. the decrease is more pronounced, 3+ or 2+ ; and finally to 0.00001, 2+ or simply +. However, there are several exceptions. The high doses of tuberculin often provoke reactions of equal intensity, but reactions more intense for a low concentration of tuberculin than for a high concentration have never been noted when the tests were made simultaneously at symmetric points of the skin.

The ecchymotic or necrotic reactions have rarely been observed among the subcutaneously vaccinated animals. More frequently, however, among those vaccinated intraperitoneally these reactions were manifest for doses of 0.01, 0.001 and 0.0001 cc. of tuberculin and occurred irregularly even at ten months after vaccination.

On account of the great individual variation and the periodic irregularities which make it useless for comparative appreciation, the intensity of the tuberculin reaction seems to us, for the present time, but an accessory pointer in the measure of the hypersensitivity to decreasing doses of tuberculin in guinea pigs vaccinated with the given doses of B.C.G.

FIGS. 1–4. *Data on guinea pigs subcutaneously vaccinated with doses of 0.010, 0.005, and 0.0025 gm. B.C.G. Variations in percentages of positive animals to an intradermal test of tuberculin for a period of four months' observation after vaccination.* FIG. 1. *Dose of 0.010 cc. tuberculin.* FIG. 2. *Dose of 0.001 cc.* FIG. 3. *Dose of 0.0001 cc.* FIG. 4. *Dose of 0.00001 cc. tuberculin.*

———— *Guinea pigs vaccinated with 0.010 gm. of B.C.G.*
— — — — *Guinea pigs vaccinated with 0.005 gm. of B.C.G.*
—o—o—o—o *Guinea pigs vaccinated with 0.0025 gm. of B.C.G.*

(*vii*). The study of the accompanying tables and percentage graphs drawn up for subcutaneously vaccinated guinea pigs, reveals certain interesting facts concerning the establishment and development of allergy among all the animals, for decreasing doses of tuberculin.

Five days after vaccination, there was an animal positive to 0.01 cc. of tuberculin among group A, vaccinated with the highest dose of B.C.G. During the course of the first ten days after vaccination, while the percentage of animals positive to this dose of tuberculin increased substantially for groups A and C, no guinea pig in group B had yet reacted. But at the end of 15 days, nearly three-quarters of the animals in each group reacted positively without great distinction of percentages among the three groups. At this period, the same animals reacted to 0.001 cc. of tuberculin for an average of about 40%; but just one animal in the three groups has reacted to 0.0001 cc. and none to 0.00001 cc., to date. There is no apparent correlation between the variations in percentages and the differences in the quantities of B.C.G. received.

At the end of the twentieth day, a good step is made in the establishment of allergy among many guinea pigs, as may be judged by the increase in percentages. It happens that the animals responding earliest to the dose of 0.00001 cc. of tuberculin, that is about the 25th day, are in group C, vaccinated with the smallest dose of B.C.G. However, two guinea pigs in groups A and B, as already mentioned, have given positive reactions for a dose of 0.000005 cc. of tuberculin at about the fourth month after vaccination.

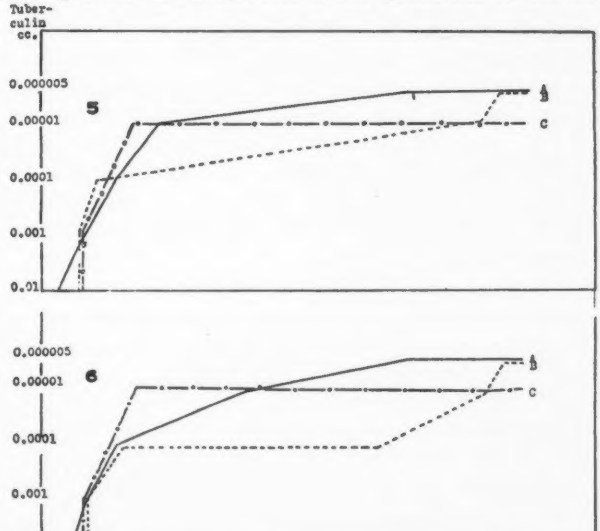


FIG. 5. *Earliest positive reactions to decreasing doses of tuberculin in each group of guinea pigs, subcutaneously vaccinated with 0.010, 0.005, and 0.0025 gm. of B.C.G.* FIG. 6. *Individual logarithmic curves showing the development of hypersensitivity to decreasing doses of tuberculin of the most sensitive guinea pig in each group of vaccinated animals.*

———— *Guinea pigs vaccinated with 0.010 gm. of B.C.G.*
– – – – – – *Guinea pigs vaccinated with 0.005 gm. of B.C.G.*
—o—o—o—o *Guinea pigs vaccinated with 0.0025 gm. of B.C.G.*

Fig. 5 expresses clearly the relation between the three groups of animals vaccinated subcutaneously, as it pertains to the precocity of the positive reactions to decreasing doses of tuberculin. Thus among these animals, the

test for 0.01 cc. of tuberculin may become positive about the fifth day after vaccination; the test for 0.001 cc., at about the twelfth day; 0.0001 cc. the 15th day; the positive reactions for 0.00001 cc. may appear at the end of the first month or during the second and third months; and finally, the test for 0.000005 cc. does not give positive results before the fourth month following vaccination.

Fig. 6 represents the curve of the most sensitive animal in each group, and illustrates how the sensitivity to decreasing doses of tuberculin may vary from one animal to another. It also demonstrates the apparent independence of the sensitivity to small variations in the dose of B.C.G. administered subcutaneously.

If one relies on the percentages, one sees that the maximum percentage of positive reactions among subcutaneously vaccinated guinea pigs is reached at about the second month after vaccination for the higher doses of tuberculin, *i.e.*, 0.01 cc., 0.001 cc., and 0.0001 cc. For the dose of 0.01 cc., the percentage reaches 100 in each group.

After this period, a certain number of guinea pigs, about 15% in each group, cease to respond to 0.01 cc. of tuberculin, and the curves of Fig. 1 come down gradually without showing any distinction between the doses of B.C.G. received. In article (*ix*) may be found the probable explanation of the sudden rise of curve C during the fourth month. During this month the sensitivity to 0.001 cc. and 0.0001 cc. of tuberculin still persists among the majority of the animals, the percentages being the same as in the second month.

The maximum percentage of reactions positive to 0.00001 cc. of tuberculin, namely an average of 48%, is seen to be reached only during the third month for groups A and C; in group B, only one guinea pig gives such a reaction and it happens during the fourth month. As already mentioned, the same fact is observed concerning the reactions to 0.000005 cc. of tuberculin which were produced in two guinea pigs not before the fourth month. We would like to call attention here to the marked delay in the appearance of the sensitivity to the minimum doses of tuberculin in many of our guinea pigs.

As already stated in article (*iv*) the tests with doses of tuberculin smaller than 0.01 cc. permit a classification of our guinea pigs as to their state of hypersensitivity.

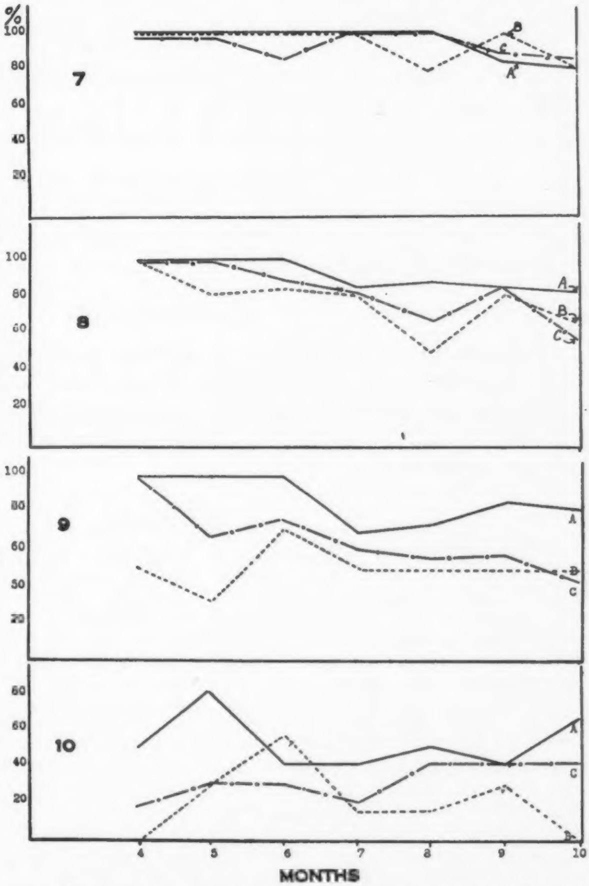
(*viii*). Concerning the animals vaccinated intraperitoneally, no other comments than the following seem justified. At the fourth month after vaccination, that is when the tests began, all the animals reacted to 0.01 cc. of tuberculin; the majority still persist in this state at the tenth month. As concerns the other doses of tuberculin, the decrease in percentages has already begun during the fifth and sixth months and continues slowly with intermittent returns.

Table III shows that, while 100% of animals react positively to 0.01 cc., 0.001 cc. and 0.0001 cc. of tuberculin, the percentage of positive reactions

to 0.00001 cc. is much lower. This last test has probably selected the most sensitive guinea pigs among which the doses of 0.000005, 0.0000025 and 0.00000166 cc. may now permit another selection. (See article (*ii*)).

Here, as among the subcutaneously vaccinated animals, the sensitivity to 0.00001 cc. of tuberculin seems retarded for many animals, since the average maximum percentage of positivity to this dose is reached during the fifth and sixth months, whereas for the remaining doses of tuberculin, the maximum was already reached during the fourth month. The group of 0.010 gm. stands out clearly from the other two, by its high percentage of positive reactions, especially for doses of tuberculin less than 0.01cc.

ix. The regression of sensitivity seems to carry an individual factor still more than its establishment, since the percentage curves decline less rapidly than they rise. In some animals, however, the sensitivity to a given dose of tuberculin is seen to disappear for a certain period, to reappear later on. These irregular recurrences explain the sudden rises of certain curves during the period of decline. This should warn us against too rapid and final a classification of the negative animals.



FIGS. 7–10. *Data on guinea pigs vaccinated intraperitoneally with doses of 0.010, 0.005 and 0.0025 gm. of B.C.G. Variations in percentages of positive animals to an intradermal test for periods of from 4 to 10 months' observation after vaccination.* FIG. 7. *Intradermal test, 0.01 cc. tuberculin.* FIG. 8. *Tested with 0.001 cc. tuberculin.* FIG. 9. *Tested with 0.0001 cc. tuberculin.* FIG. 10. *Tested with 0.00001 cc. tuberculin.*

——————— *Guinea pigs vaccinated with 0.010 gm. of B.C.G.*
— — — — — — *Guinea pigs vaccinated with 0.005 gm. of B.C.G.*
—o—o—o—o *Guinea pigs vaccinated with 0.0025 gm. of B.C.G.*

Sufficient data to make any more comment on the regression of sensitivity are not yet available. Measurement of the length of its persistence for all doses of tuberculin is now planned.

Summary

1. A quantitative study of the allergic state of guinea pigs vaccinated with 0.010, 0.005 and 0.0025 gm. of B.C.G. has been sketched as a preliminary to researches on their state of immunity.

2. By serial and intradermal reactions with decreasing doses of tuberculin, the measurement of the degree of allergy of these animals at different periods after inoculation has been undertaken, as has also the determination of the development of this hypersensitivity.

Acknowledgment

We wish to offer our thanks to the National Research Council of Canada and the Faculty of Medicine of the University of Montreal in the person of Dean Dr. Telesphore Parizeaü, for their hearty encouragement. Our special thanks are also due to Dr. T. I. Hoen, a colleague at St. Luke's Hospital, for his aid in the English transcription.

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THE LIFE CYCLE OF *FASCIOLOIDES MAGNA* (BASSI, 1875), THE LARGE LIVER FLUKE OF RUMINANTS, IN CANADA

WITH OBSERVATIONS ON THE BIONOMICS OF THE LARVAL STAGES AND THE INTERMEDIATE HOSTS, PATHOLOGY OF *FASCIOLOIDIASIS MAGNA*, AND CONTROL MEASURES[1]

By W. E. Swales[2]

Abstract

The life history of *Fascioloides magna* in Canada has been elucidated, the intermediate hosts being two fresh-water gastropods, *Fossaria parva* (Lea) and *Stagnicola palustris nuttalliana* (Lea). The morphology and bionomics of the egg and larval stages are described, particular attention being given to the non-parasitic stages. The ecology of the gastropod hosts in Canada is briefly described.

A histopathological study of the lesion in definitive hosts reveals that this parasite in large Bovidae causes a severe tissue reaction. The lesion in these animals is generally in the form of a closed fibrous cyst from which eggs are unable to pass, and thus the life cycle cannot be completed. In Cervidae, the cavity in the liver is connected directly with the bile duct system, and there is a free egress of ova. From these facts it is inferred that this parasitic disease can only occur in the presence of Cervidae.

Laboratory animals have been artificially infested with maritae, thus extending the host records of the trematode.

A brief historical review, a summary of the present knowledge of distribution and definitive hosts affected, and a description of the control measures, are included.

Introduction

Fascioloides magna, the digenetic trematode which forms the basis of this study, is essentially of North American origin but has been introduced into Europe in imported animals. It is an extremely important helminth parasite of ruminants both from a pathological and economic viewpoint. It is unlikely that it will ever be as widespread in its distribution as *Fasciola hepatica*, with which it has so often been compared, but it has been described as being of equal importance in some parts of North America.

In spite of being native to North America this trematode was first described by Bassi from various deer enclosed in a national park in Turin, Italy. It is generally agreed that the Italian epizootic was the result of importations of American wapiti. The parasite had, however, encountered a satisfactory intermediate host in Europe because several ruminants of other species were infested at that time and it has since been reported from southern Germany. Owing to its peculiar location in the liver tissue of its host and the outward similarity of the lesion to a common abscess, it is probable that it has been overlooked in many cases.

[1] *Manuscript received December 26, 1934.*

Contribution from the Institute of Parasitology, McGill University, Macdonald College, Que., with financial assistance from the National Research Council of Canada.

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This parasite was the subject of an extensive morphological study by Stiles in 1894. In this work the author included an excellent historical review of the species and elucidated its synonymy. Stiles also included notes on the pathological effect upon the definitive host and gave a comparative description of the egg and miracidium. He stated that the life cycle was probably very similar to that of *F. hepatica* and experiments were being conducted at that time in order to determine the different stages in an intermediate host. It was not until very recently, however, that an intermediate host was reported by Sinitsin (1930) but now three more experimental snail hosts have been recorded by Krull (1933), the latter during the later stages of this work.

Hadwen (1916) and Cameron (1923) recorded this fluke from ruminants in British Columbia and Alberta respectively. The records of the National Parks Branch of the Department of the Interior of Canada revealed in 1931-32 that infested ruminants in the Wainwright area of Alberta were becoming more numerous. With the co-operation of the National Parks Branch and the Health of Animals Branch, the writer undertook a survey of the endemic area and made preliminary investigations into the life cycle. It was then noted that the snail fauna was peculiar in that very few members of the group recorded as hosts for other fasciolinid trematodes were present. No naturally infested snails were obtained although it seemed probable that one snail (*Stagnicola palustris nuttalliana* Lea) was a natural host.

Owing to the advanced season and the limited time available, further investigations were confined to records of the prevalence of the parasite, the determination of ecological observations upon the definitive hosts and the practicability of future control measures on the observed area.

Following the establishment of the Institute of Parasitology at Macdonald College in 1932, it was decided to continue the investigations. In 1933 and 1934, with the whole-hearted support and co-operation of the National Parks Branch, the life cycle of this trematode on the endemic area was elucidated and a fairly complete study of the bionomics of the parasite was completed. Thereafter, control measures were applied to the area most affected, and it is confidently expected that this parasite will be kept under control in the regions of heavy infestation.

Historical Review and Notes on Distribution and Definitive Hosts

To present a full historical review of *Fascioloides magna* is merely to repeat Stiles' excellent account in his paper on the anatomy of this parasite. However, for the benefit of those to whom Stiles' paper is not now available, a brief historical summary taken chiefly from Stiles, but with a few additional notes, is given here.

Bassi, in 1875, observed an epizootic among deer of the Royal Park near Turin, Italy, the symptoms being typical of the well known fascioliasis in sheep. He gave an unsatisfactory description of the parasite present, naming

it *Distomum magnum*. He believed that it was introduced into the park in American Wapiti (*Cervus canadensis*). Most workers did not accept Bassi's species because of his poor description, but it was accepted in 1882 by Perroncito who described it as "*Distoma grande*, (*Distoma magno*, *Distoma magnum* Bassi)."

In 1887, Curtice recorded this trematode in cattle in Kansas, U.S.A., and while at first identifying it as *F. hepatica*, later said it was *F. magna*.

From 1889 to 1892, this parasite was recorded from the United States by Dinwiddie, Osborne (1890), Curtice, Francis (1891) and Leidy. Hassall, 1891, described it as a new species, under the name *Fasciola carnosa*, but finding the specific name already applied to another distome, changed it during the same year to *Fasciola americana*. In the same year, Francis described it as *Distomum texanicum*, being advised by Leidy who also had overlooked Bassi's paper. Leidy himself later considered the form as identical with *Distoma crassum* which he had found in the intestine of a Chinese boy; this was later shown to be quite a different species.

Stiles, in 1892, pointed out that Francis' species was identical with *F. americana* Hassall, and was probably the parasite originally described by Bassi; he later compared specimens and proved this hypothesis. Just before this Leuckart compared an American specimen with one of Bassi's original specimens and declared them identical. Stiles (1891) had shown that the specific name *magna* should be retained for Bassi's species, and thus the Italian specimens reported by Bassi, Perroncito and Sonsino (1890) and the American species described by Hassall and Francis were henceforth recognized under the name *Fasciola magna* (Bassi, 1875).

In 1917 Ward showed that, owing to the lack of the distinct anterior cone and the fact that the vitellaria are confined to the region ventral to the intestinal branches, the suggestion of Odhner that this form should constitute a new genus should be followed. He therefore named a new genus *Fascioloides*, designating as the type *Fascioloides magna* (Bassi, 1875) Ward, 1917.

The synonymy is now:—

Distomum magnum Bassi, 1875.
Distomum hepaticum Curtice, 1882.
Fasciola hepatica Dinwiddie, 1889. (Nec. Linn. 1758).
Fasciola carnosa Hassall, 1891.
Fasciola americana Hassall, 1891.
Distomum texanicum Francis, 1891.
Cladocoelium giganteum Stossich, 1892.
Fasciola magna (Bassi, 1875) Stiles 1894.

As stated elsewhere in this paper, the fact that the external appearance of the liver lesion caused by *F. magna* is totally unlike that caused by other liver flukes, may account for the scanty records in some parts of North America. Owing to the danger of contaminating equipment, large abscesses in the liver of cattle are not incised during meat inspection and this fact would account

for the scanty number of records in Canadian cattle. The fact that this fluke is of such prevalence and importance in cattle in the southwestern United States, indicates that it is at least a great potential danger to Canadian cattle. It is common in many wild and semi-wild ruminants in Alberta and British Columbia, and this fact indicated that it only needed the introduction of the ova into many cattle-raising districts to produce conditions similar to those described by Stiles in the United States.

In British Columbia, reports issued from time to time indicate that deer on the coast are badly affected by liver fluke disease. Mr. F. R. Butler, Inspector of the Game Commission of that province, informs me that there is from time to time mortality among deer, principally on Vancouver Island, as a result of liver fluke. Many deer from these regions have been examined by authorities, and specimens of liver flukes, all of which are *F. magna*, have been forwarded to me. This was the locality of the first recorded case in Canada, and it is apparent that the reports from this district at least indicate the prevalence of the trematode in the province.

Records of distribution in the United States, including those summarized by Hall in 1912, show that *F. magna* occurs in the states of Texas, Kansas, Arkansas, California, Illinois, Iowa, Minnesota, Michigan, Montana, Oklahoma, Wisconsin, Colorado (?) and New York. In Canada, records already published indicate that the fluke is confined in distribution to British Columbia and Alberta. However specimens from *Odocoileus virginianus* in eastern Ontario have recently been received. This record considerably extends the distribution in Canada, but it is to be expected that the parasite will be recorded from other provinces.

The recorded definitive hosts are as follows:—

UNITED STATES OF AMERICA			
Cattle	(*Bos taurus*).	Sheep	(*Ovis aries*).
Wapiti	(*Cervus canadensis*).	(?) Goat	(*Capra hircus*).
Moose	(*Alces alces americana*).	Horse	(*Equus caballus*).
Deer	(*Odocoileus virginianus*).		
ITALY			
Sheep	(*Ovis aries*).	Fallow deer	(*Dama dama*).
Wapiti	(*Cervus canadensis*).	Stag or Red deer	(*Cervus elaphus*).
Blue bull	(*Boselaphus tragocamelus*).	Sambur	(*Cervus unicolor*).
GERMANY			
Red deer	(*Cervus elaphus*).		
CANADA			
Bison	(*Bison bison*).		
Coast deer	(*Odocoileus columbianus*).		

to which I am able to add:—

Yak	(*Bos grunniens*).	Virginia deer	(*Odocoileus virginianus*).
Hybrid, Domestic × Bison	(*Bos taurus* × *Bison bison*).	Mule deer	(*Odocoileus hemionus*).
Cattle	(*Bos taurus*).	Wapiti	(*Cervus canadensis*).

In addition to the above-mentioned naturally infested hosts are the following which were artificially infested during the course of this study.

Sheep	(*Ovis aries*)
Domestic rabbit	(*Lepus cuniculus* var. *domesticus*)
Guinea pig	(*Cavia porcellus*).

Neveu-Lemaire refers to *F. magna* as a parasite of cattle, sheep and goats, but the reference to the last host is obscure. At least one obscure record of this parasite in goats in the United States is present in old literature, but an uncertainty exists as to the origin and identification of the specimens. There is little doubt, however, that *Capra hircus* could be infested.

Snail Hosts with Notes on Their Ecology

In order to determine the intermediate host of *F. magna* in Canada an endemic area in Alberta was selected and a thorough survey of all the aquatic and semi-aquatic gastropods was undertaken. This area is situated in the vicinity of Wainwright and comprises approximately six thousand acres; it is part of a National Park and its fences enclose herds of *Bison bison*, *Bos grunniens* and *Cervus canadensis*. The young animals of the last-mentioned herd were heavily infested with the trematodes and have had access to no other grazing lands; many showed clinical symptoms of fascioloidiasis.

The area is typical "Western parkland", rolling prairie with sandy soil and scanty tree life composed of scrub poplar bluffs. A lake, about one square mile in area, is situated near the centre of the main enclosure; this is fairly "alkali", has well-defined sandy shores and harbors little or no vegetation and hence no snails could be found in its environs. One other permanent lake is present which is not one of the so-called alkali lakes. It covers approximately one hundred acres, is surrounded by a shore-line rich in vegetation and contains a snail fauna of four species, *Stagnicola palustris nuttalliana*, *Lymnaea stagnalis wasatchensis* and two *Gyraulus* spp. The water in midsummer is only slightly alkaline (pH 7.5–8.0).

The other watered areas are 32 small swamps, muskegs and sloughs, only six of which are permanent, and a small sandy-shored lake which contains no snail life. The water in the above-mentioned areas varies in alkalinity from pH 8.5 to pH 9.5 and is stagnant. The permanent, and some of the semi-permanent, swamps and sloughs were rich in snail life composed of the following species, listed in approximate order of prevalence.

1. *Stagnicola palustris nuttalliana* (Lea).
2. *Aplexa hypnorum tryoni* Currier.
3. *Lymnaea stagnalis wasatchensis* Hemphill.
4. *Physa gyrina* Say.
5. *Succinea retusa* Lea. (On surrounding soil.)
6. *Gyraulus circumstriatus* (*Tryon*).
7. *G. umbilicatellus* (*Cockerell*).
8. *G. deflectus obliquus* (DeKay).
9. *G. crista* (Linn.)
10. *Helisoma trivolvis* (Say).
11. *H. subcrenatum* (Carpenter).
12. *Menetus exacuous megas* (Dall).

S. palustris nuttalliana is by far the most prevalent of the snails present and is the only species found in three sloughs which were observed for three consecutive years and which were completely dry in mid-July of each summer. Often more than fifty specimens of this snail have been collected with one three-foot sweep through the vegetation with a five-inch scoop.

In addition to these, there are four small areas where there is a spring-time accumulation of water which is never of great volume but is contained in an assembly of small depressions amid seepy land and is covered by sedges (*Carex* spp.). These areas altogether do not comprise more than two acres, three of them are natural drainage ways leading to a permanent slough or lake in each case. They are completely dry in the second or third week of June in normal years and, during preliminary investigations, were not considered as possible snail habitats owing to the fact that this work was undertaken in July. Later investigations showed that, in May, these areas harbored *Fossaria parva* (Lea) in small numbers. These snails were found in animal tracks effectively covered by sedges and were difficult to find. They were feeding on dead sedge leaves and at times were found to be actively crawling over the mud sides of the tracks. The largest population observed was fifty snails on an area of approximately 200 sq. ft.

Cercariae of *F. magna* emerged from one naturally infested specimen of *F. parva* on the twentieth day after collection, and from four other specimens of this species on the twenty-fourth day. From all of these snails experimental definitive hosts were successfully infested with maritae. Following these findings, particular attention was paid to the habitats of *F. parva*. Towards the end of July an attempt was made to determine the level to which the snails had burrowed when their habitat dried up. It was found that young snails could be recovered from the first two six-inch layers of the soil over their spring habitat. The majority appeared to be at a depth of six inches, under soil which was well covered with dried sedges. These observations were made by a co-operator, on behalf of the writer, and were not sufficiently extensive to indicate definitely the means employed by these animals to withstand adverse conditions. It is probable then, that the cycle of the trematode within the snail must be completed under natural conditions in the short period between the thawing and drying of the upper soil of the areas under study in Alberta. Alternatively, the intramolluscan stages of the parasite may live over the dry summer months and the winter in the tissues of the snail, their activity and development corresponding to that of their host.

In addition to the *F. parva*, specimens of all the other snails were returned to the Institute and kept under observation in aquaria. Cercariae emerged from one naturally infested specimen of *Stagnicola palustris nuttalliana* on the sixty-ninth day following collection. Nine of the encysted metacercariae were fed to a rabbit which was successfully infested with two maritae of *F. magna*. This snail was one of 400 kept under observation, the remainder being negative on subsequent examination.

No other species of snail was found to be naturally infested; artificial infestation was also unsuccessful in spite of the fact that miracidia readily attacked *Aplexa hypnorum tryoni*, *Physa gyrina* and *Lymnaea stagnalis wasatchensis*.

Of 50 specimens of *F. parva* kept under observation, five were found to be naturally infested. From one of these specimens 687 cercariae emerged and encysted during a period of 58 days.

Two species of snails were thus shown to act as natural intermediate hosts for *F. magna* in Canada. *F. parva* (Lea) was found to be living in small areas which are wet and swampy in the spring time but are completely dry during summer and autumn. It apparently burrows into the soil in order to survive the unfavorable dry seasons. It feeds on the limited amount of decaying vegetation, composed chiefly of sedge leaves and grass. This species is very active, and is often found on the mud surrounding water-filled animal tracks which contain most of the free water in May.

S. palustris nuttalliana (Lea) is very different from *F. parva*, both from the ecological and morphological points of view. It is at present recognized as a sub-species of *S. palustris* (or *Lymnaea palustris*, under which name it is still recognized in some parts of the world), but may be a geographical strain. It inhabits stagnant bodies of water which contain large quantities of vegetation, both living and dead. These waters may be permanent or semi-permanent, and on the endemic area are always fairly warm and have a large range of alkalinity. Sloughs that dry up in the spring are not favorable habitats, but those that contain water until midsummer are very suitable. It is often found in company with *L. stagnalis wasatchensis* Hemphill, in small lakes containing abundant vegetation. Unlike *F. parva* it is not found out of the water, and prefers to remain under the surface of its stagnant water habitat. Although only one specimen of this snail has been found to be infested under natural conditions, it can easily be infested artificially, and is able to act as an intermediate host throughout the cycle. It is probable that examination of a great number of specimens from an infested area would reveal a number of naturally infested individuals.

The inclusion of *S. palustris nuttalliana* in the group of Lymnaeidae known as intermediate hosts of fasciolinid trematodes in North America is important. It means that snails of this universally prevalent species must be considered as possible vectors of *F. magna*, even where snails of the other groups have been destroyed. Control measures must always be modified accordingly, but more strict attention must be given to the warning of Baker, that promiscuous applications of copper sulphate may have untoward effects. This snail also lives normally in lakes inhabited by fish ot several species and in waters suitable for fish spawning grounds.

In addition to the snails shown in this work to be intermediate hosts in Canada, are *Galba bulimoides techella* (Halderman), *Fossaria modicella* (Say), *F. modicella rustica* (Lea) and *Pseudosuccinea columella* (Say). The first one, which has not yet been recorded in Canada, was reported by Sinitsin as a host in Texas, U.S.A., and the other three recently reported as artificially infested species by Krull (1933) in the United States.

G. bulimoides techella, *F. modicella* and *F. modicella rustica* are commonly found in intermittent pools or streams and have not been recorded in habitats similar to those here described for *F. parva*. They may be found in company with *S. palustris nuttalliana* but the more common habitat of this snail would

not be suitable for their common existence. These three species were not present on the small endemic area under study, but *F. modicella* was found in small numbers in a spring-fed pool about seven miles away.

P. columella is known as a "quiet bay or pond type" and is found in company with *L. stagnalis*. It was not present in the Alberta area but it may be found in the same habitat as *S. palustris* (or *Stagnicola* spp.) in other areas.

The Intermediate Hosts in Aquaria

F. parva was kept in shallow nine-inch specimen jars in two to three inches of tap water. Dried sedge leaves, moss and filamentous algae were added, and dried lettuce leaves were supplied as food. The water was completely changed twice per month but fecal matter was siphoned off twice a week. Sand and mud were supplied to two jars but this apparently made no difference to the health of the animals. Air was continually bubbled through the water in each jar by means of a series of glass tubes connected to a German K D A air pump.

A second generation of this species was observed after forty days in the aquaria and these specimens were used for artificial infestation experiments, when they were over four days old. Even when snails of these species were extremely small they were able to withstand infestations and to produce cercariae normally. No death from the effects of the parasites has ever been observed, which leads to the belief that this species is the normal intermediate host in Canada.

S. palustris nuttalliana was kept in large jars which were aerated as already mentioned, but were only cleaned once in two months, tap water occasionally being added to replace that lost by evaporation. These snails quickly removed growing algae if in large numbers, but did not appear to suffer from its consequent absence. They were fed on dried lettuce leaves and dried sedges, and consumed large quantities of the former when it was supplied in a dried condition which speeded its decomposition in the water.

A second generation of these snails did not appear until the adults had been in the aquaria for 65 days. In the first attempts to infest this species many of the snails died on the third and fourth days, but this phenomenon did not re-occur in further tests, so that it is probable that some other factor lowered the resistance of the snails.

A factor that was at first considered important was the hydrogen ion concentration of the water in aquaria. Tests of water in aquaria showed that *F. parva* lived normally in water ranging from pH 6.5 to pH 8.5 and that *S. palustris nuttalliana* lived in waters ranging from pH 5.5 to pH 9.5. These figures agree on the alkalinity side with the findings in their natural habitat. The pH of the water in the aquaria was subsequently never corrected, and there were no ill effects upon the animals.

It was found desirable to add a small lump of calcium sulphate to each tank in order to supply the calcium requirements of the snails. This apparently increased the growth rate of the young snails.

The shell of laboratory-raised specimens of *F. parva* is somewhat darker in color than that of the snails in their natural habitat. On the other hand, the shell of *S. palustris nuttalliana* becomes quite light in color after about one month in the tanks.

The temperature of the general aquarium room could not be accurately controlled, but the mean temperature throughout the experiments was 24°C.

Artificial Infestations

For the purpose of determining the time required for completion of the intramolluscan stages in *F. parva*, five embryonated eggs per snail were suspended in a small vial in the jar containing the snails under experiment. In this way, the hatching time and hence the approximate time of infection was determined by frequent examination of the ova in the vial. In the first two tests the twelve snails in each were all infested and the cercariae commenced to emerge on the forty-ninth and fifty-fifth day, respectively. On the day of the first appearance of metacercariae on the walls of the test jars, each snail was placed in a separate jar and the date of cercarial emergence was noted. The periods ranged from 49 to 58 days from the day of miracidial attack.

Second generation *S. palustris nuttalliana* were exposed in a similar way, but in the first three lots of 12 snails the development of the sporocyst did not appear to continue after the fourth day. In one other lot of ten snails, eight specimens subsequently produced cercariae and the first encysted metacercariae were noted on the walls of the tank on the fifty-seventh day.

Pre-parasitic Stages in the Life Cycle

The Egg

The eggs of *F. magna* are passed out of the normal fluke cavity in the host's liver tissue by way of the small bile ducts, which are merely intercepted in their course and are not closed off by the formation of the normal fluke cavity. From here they progress normally, passing into the alimentary canal by way of the main bile duct. In long standing cases, or in heavy infestations, some of the fibrous cavities are completely cut off from the bile system and are then non-functional. No infested Bovidae have been found to be evacuating eggs in the feces. One animal (*Bos grunniens*) was found to be infested with 12 adult flukes in completely closed fibrous cavities and had 24 other cavities in which only dead and decomposed flukes were observed. Of all the eggs which were present in a heavy suspension in the melanoid fluid, less than 0.1% developed in culture. In one *Cervus canadensis*, which harbored 15 live flukes in nine "open" cavities and two "closed" cavities, eggs were passing out of the main bile duct in huge numbers, the formolized duct yielding 497 eggs per millimetre of length.

One other test animal (*Cervus canadensis*) which harbored 20 live adult flukes, all in normal open cavities, was found by four tests to be passing an average of 37.41 eggs per gm. of feces. Only one adult animal of this species could be confined for the purpose of obtaining the number of eggs per day per fluke but rough estimations indicate that this figure would be between six and seven thousand.

These findings do not agree with those of Sinitsin, who on two occasions has stated that the eggs probably pass in some unexplained way into the blood stream, because he has found no communication between fluke cavities and bile ducts. It is apparent that Sinitsin was working with animals that had reacted to the infestation to such an extent that the fluke cavities were closed by a complete fibrous cyst. This view is supported by the fact that he was working with *Bos taurus*, obviously an abnormal definitive host for *F. magna*, which, it is generally agreed, is of North American origin. The writer has noticed this condition on two occasions in *Bos grunniens*, but has always found a heavy egress of eggs in *Cervus canadensis* and *Odocoileus* spp.

Morphology of the Undeveloped Egg

The size and shape of the ova vary considerably, the most usual specimen being 148μ long by 94μ wide and ovoid in shape. In order to indicate the variations in size more clearly than is possible by giving extreme and average measurements, the length and width curves from the measurements of 200 ova were plotted (Fig. 1). These were taken from a mixed lot of fresh feces of *Cervus canadensis*, and the figures obtained agree closely with all the writer's previous findings. It will be noted that the variation in length is much greater than in width, and that greater abnormalities are liable to occur in the length. The normal egg size as indicated by the chart is not the average size.

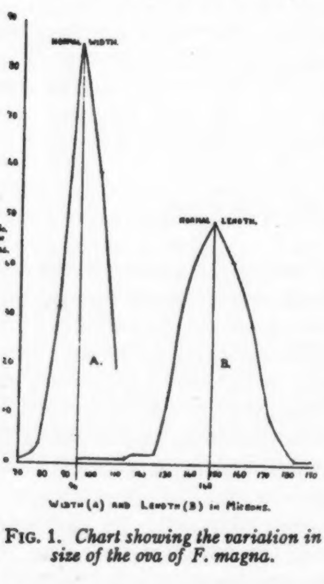

FIG. 1. *Chart showing the variation in size of the ova of F. magna.*

Eggs containing fully formed miracidia vary very considerably in shape; probably the activity of the miracidium causes lateral distention or elongation in many cases. It has been noted in many cultures, kept in deep stagnant water to prevent hatching, that the eggs tend to become broader. Many of these appear similar to the abnormal shapes of the eggs of *Fasciolopsis buski* as pictured by Barlow (1925).

The size of mature eggs teased from the uteri of live flukes does not appear to vary in relation to the size of the trematode. The developmental period of these eggs is the same as that of eggs freshly passed from the host's alimentary canal.

The peculiar appendage on the anti-opercular end of the egg as described by Sinitsin (1933) is present in varying forms on practically all eggs removed from the fluke cysts in the liver. It is only present on approximately 20% of eggs passed normally from the host. This appendage protrudes from a small pore which is situated slightly to one side of the middle line of the egg; it varies from 4 to 21μ in length. The findings of the writer indicate that it is an irregularly shaped mass of protoplasm which has been extruded through the pore, probably by some pressure within the egg while it is in the uterus of the trematode. The appendage is often lost if the eggs are forcibly screened through fine sieves, which may explain why this feature was not noted by Stiles (1894) in his description of the egg.

The writer cannot agree that the eggs of *F. magna* are lighter in color than those of a *Fasciola*. The eggs of *F. hepatica* are aptly described as being of a delicate light brown color; those of *F. magna*, normally evacuated from the host, are golden brown and somewhat more opaque. They are easily distinguished by this darker coloration from normally evacuated eggs of *F. hepatica* of sheep, as well as by their larger size and by the presence, in some of them, of the afore-mentioned appendage. In addition to these distinguishing features there is a marked thickening of the shell at the anti-opercular end, varying from one and a half to three times the thickness of the other parts of the shell. The operculum is irregularly rounded and from 15 to 20μ in diameter.

Freshly evacuated eggs have an undivided germ cell and approximately forty yolk globules which are somewhat more opaque but otherwise very similar to those of *F. hepatica* and to the descriptions of *F. buski*. The germ cell is difficult to observe, being smaller than that of *F. hepatica*. After the eight-cell stage, the development is even more difficult to follow until the morula appears; however, no observations have been made that show any difference in comparison with the other closely related trematodes.

The miracidium develops quickly, cilial motion being observed some time before the eye spot appears. The mucoid plug grows rapidly until the miracidium has reached its full development, by which time yolk globules have been broken up and the oily substance has collected into two masses.

The miracidium presents a cramped appearance in the egg, being larger than in related species. The anterior end is abruptly curved over between the first and second quarter of the body's length. In the animal's struggles at hatching time the anterior end is straightened and the mouth is directed into the mass of the mucoid plug, which in this case is readily flexible. The body then takes a rough "V" shape, and the oily masses are displaced.

Development of the Egg

The eggs commence to develop as soon as they are passed into suitable conditions of temperature and moisture. In the summer time in western Canada they are ready to hatch in 35 days, even though they remain in damp feces. Many of the eggs taken from "closed" cysts in the liver do not develop at all, or if they do, they take much longer than normally evacuated ones.

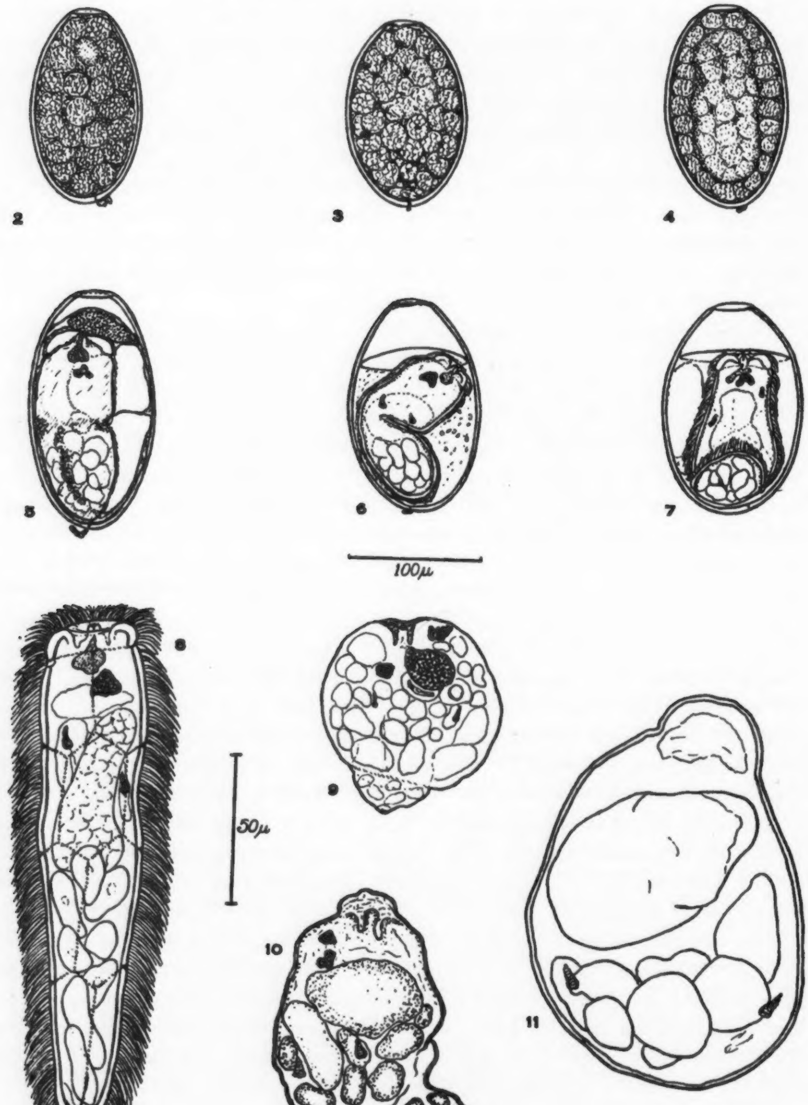

FIG. 2. *An ovum in the one-cell stage showing the germinal area and the arrangement of the yolk balls.* FIG. 3. *An ovum in the 16-cell stage.* FIG. 4. *The appearance of the morula.* FIG. 5. *An immature miracidium in an ovum on the twelfth day of development in water.* FIG. 6. *A mature and very active miracidium in an ovum after 30 days in moist feces.* FIG. 7. *Position taken by the miracidium immediately prior to hatching.* FIG. 8. *A miracidium in the position taken when swimming in water.* FIG. 9. *A 12-hour sporocyst.* FIG. 10. *A 48-hour sporocyst.* FIG. 11. *A sporocyst from the pulmonary sac of a snail, 120 hours after miracidial attack.*

Sinitsin did not observe miracidia in the eggs before the thirty-third day and he notes that the cysts with which he worked had no exit via the bile system. These facts indicate that he was using eggs that had been held at body temperature for an abnormally long time and thus they cannot be considered as normal eggs of *F. magna*.

The development of the eggs in water or feces is not synchronous. At a mean temperature of 24° C. (maximum 27° C., minimum 21° C.) the majority of eggs in a water culture reach the morula stage in ten days. In one culture under three inches of water in a beaker, at the above-mentioned temperature and with the water changed daily, hatching commenced on the twenty-ninth day. These continued to hatch in numbers until the forty-fifth day.

Freshly evacuated eggs in the one-celled stage do not develop further during storage at a temperature of 2 to 5° C. Continual storage at this temperature up to at least a period of nine months, does not retard subsequent development. If the eggs at a late stage of development are frozen in water, they are promptly destroyed, although the miracidium in fully developed eggs may be slightly active for some hours after thawing. This activity has been observed in eggs after they have been frozen in water for 12-, 24- and 36-hr. periods, although the epithelial cells are in all cases badly damaged and the miracidium is incapable of swimming when artificially released from the damaged shell. Ova in the one-cell to the 16-cell stage are not readily affected by freezing in water or moist feces. A mixed lot of eggs was frozen at −5° C. in water and in feces for periods up to six weeks. Almost all the eggs which were at a stage less developed than the morula, were not affected, and developed normally when removed to laboratory temperatures. More detailed studies on the effect of cold had to be postponed owing to a seasonal difference in the rate of development.

The greatest factor in stimulating rapid development is temperature. Although development has not been studied under many different, controlled temperatures, it seems certain that the optimum is between 24 and 28° C.

On several occasions undried feces, which had been protected by vegetation and which contained eggs with active miracidia, have been found in the field. These eggs hatched normally and have been used in tests of possible intermediate hosts. Studies were subsequently made of this unusual development, using feces of *Cervus canadensis* which, when fresh, had a moisture content of 76-80%. Lots of fresh feces were placed in chambers and the moisture estimated by weighing portions, baking them for three hours at 56° C. and then reweighing. The samples at this time were hard and dry in appearance and to the touch. One lot, approximately 200 gm. in weight, was placed in a "moist-chamber" dish at 24° C., and the moisture gradually reduced by removing the water of condensation from the cover and sides daily. Some of the contained eggs were ready to hatch on the thirty-sixth day, when the moisture content of the feces was 61%. Other samples were prepared for observation by placing smaller amounts of fresh feces in three-inch glass-stoppered specimen jars and the moisture was reduced to a different degree

in each lot by exposing them to a dry atmosphere for various periods. After the jars had been sealed for 11 days the percentage of moisture by weight was calculated. No. 1 contained 76% moisture while Nos. 2–6, contained 73, 72, 66, 72 and 60%, respectively. In subsequent examination there appeared to be no difference in development, and on the thirty-fifth day after collection all the feces in each lot were sedimented and the eggs removed. In each lot about 40% contained active miracidia, 50% were in the morula stage and the remaining 10% were either destroyed or undeveloped. As this work was conducted in the autumn when other routine cultures were, for some unexplained reason, developing very slowly, further investigation was discontinued.

The above observations merely demonstrate that feces which fall into shaded places must be considered as possible media for viable eggs which can hatch during the course of a rainstorm or a flood. It is very probable that snails migrating from their habitat during a rainstorm are infested by miracidia which have suddenly hatched after having developed in feces.

The eggs are rapidly destroyed by complete drying, or by the reduction of moisture to a point below 50%. From these findings it is certain that eggs evacuated in feces and subsequently exposed to the full rays of the sun, will all be destroyed in less than three days.

Hatching

Eggs hatch sporadically, the phenomenon not being affected by light. Although most swarms of miracidia have emerged from the cultures at night time, hatching has frequently taken place under a strong artificial light. The results of attempts to determine the effects of darkness and light have been contradictory and the writer is convinced that the light factor plays little or no part in the process.

Attempts were made to stimulate hatching by controlling atmospheric pressure in a glass-topped receptacle. The eggs were placed in staining dishes such as are used for routine collection of miracidia and were carefully watched for periods ranging from one-half hour to one hour while under pressures varying from 25 to 33 in. No hatching was noted at any of these pressures or during any change in pressure, although nearly all the ova hatched normally following the experiments. These tests were conducted at various times of the day and evening.

Sudden changes in temperature will often accelerate hatching. Eggs taken from the refrigerator at 3° C. will frequently hatch in less than an hour in the laboratory at 22-24° C. This method is, however, far from infallible. Conversely, eggs will occasionally hatch on being moved from the laboratory to the refrigerator. One other factor controlling hatching is the aeration of water cultures. Eggs in stagnant culture dishes will remain unhatched for periods ranging from one to five months but when placed in fresh tap water or aerated water from aquaria, will soon hatch normally.

It is apparent that the phenomenon of hatching is controlled not by one, but by several factors, the temperature and aeration of the water playing the greatest parts.

In hatching, the miracidium straightens its head and pushes vigorously against the mucoid plug, with cilia lashing rapidly. It then attempts to straighten its whole body and, if successful, the operculum springs off or turns up as if on a hinge, the mucoid plug flows out and ruptures the vitelline membrane. The miracidium follows rapidly and swims away. One other phenomenon occasionally observed is hatching posterior end first: the miracidium turns completely around and, instead of pushing the plug with its anterior extremity, applies its exertions to the anti-opercular end. The operculum opens as before and the miracidium emerges, tail first, very much more slowly than it does by the more normal method because of the posteriorly directed ciliae. At the time the miracidium has emerged in this way as far as its head the body is stretched to a length approximately three times that of the egg.

The anti-opercular pore appears to play a part in the process of hatching by helping to offset the low pressure in the eggs caused by the exit of the mucoid plug, vitelline membrane and miracidium.

Snails eat eggs of *F. magna* very readily and it was thought probable that they become infested in this way. However, six snails placed in a culture from which miracidia were ready to emerge later evacuated eggs which were all unhatched. Since this simple test, embryonated eggs have been observed in snail feces upon many occasions and it is improbable that hatching is accelerated by the ingestion of the egg by the snail. Owing to the fact that the alimentary tract of the snail is very active and that evacuation of waste ingesta takes place soon after the food is swallowed, it is unlikely that infestation can result from this habit.

The Miracidium and Its Attack upon the Snail Hosts

Upon hatching, the miracidium swims in the surrounding water at a speed not approaching that of the miracidium of *F. hepatica*. Its speed has been estimated at 4 mm. per sec. by observing numerous specimens swimming over a scale on the stage of a dissecting microscope.

The miracidium can readily be studied in an egg albumen mount, but the portion of egg albumen on the slide should be allowed to dry around its circumference before placing the miracidium in the centre and applying a cover slip. This slight modification of Krull's method of using egg albumen as a mounting medium for larvae is more satisfactory for miracidia in that it prevents undue contraction of the body, owing to the increased viscosity.

In the normal swimming position the mean measurements of the miracidium are as follows: 0.211 mm. long by 0.032 mm. wide at a point immediately posterior to the eye spot. The head cilia are 10μ long; the cilia at mid-body

are 12μ and those at the posterior extremity are 16μ in length. The head collar is 0.023 mm. in diameter when in this position. The two flame cells appear to be 10μ long and are situated 81μ and 89μ from the anterior extremity.

Except in size the organism is very similar in general morphology to other fasciolinid miracidia.

The miracidia quickly die in water that has remained standing in the laboratory for some days, but will swim actively for 12-24 hr. in fresh tap water or in water from aerated aquaria. They do not appear to be attracted to either direct or diffused light; a strong microscope light does not affect them in any way.

The miracidia almost always swim in swarms and exhibit a similar appearance and the same activities as described for *Fasciolopsis buski* by Barlow.

The Attack upon the Intermediate Hosts

As described under snail hosts, the organisms do not show a distinct preference for their natural hosts, but readily attack several other gastropods. The strong chemotaxis described by Barlow (1925) for *Fasciolopsis buski* has not been observed. The miracidia will usually swim several times around a staining dish containing a suitable snail host before attempting to enter it. Some of them make futile attempts to penetrate the head and foot but the successful ones eventually find their way under the mantle fold and penetrate the posterior part of the pulmonary sac. For this reason the actual process of penetration into the host's tissues is rarely observed and some of the attempts to infest a snail with a single miracidium have failed owing to the uncertainty of penetration in spite of careful observation. From the study of the next stage in the cycle it appears that the penetration and the casting of the cilia and epithelial cells takes place as in related species.

The attack by miracidia does not appear to cause the snail any discomfort judging from its placid progress over the surface of the infection chamber even when many larvae are attempting to penetrate its tissues.

The Sporocyst and Rediae

Sinitsin (1933) describes a rather sensational method of parthenogenetic reproduction by the intramolluscan stages of *Fasciola* spp. He describes a phenomenon of "egg-laying" by the primary sporocyst or "euparthenita," the "eggs" developing into secondary sporocysts or secondary euparthenitae. These produce a great number of mother rediae or "migratory pseudoparthenitae" which migrate towards the liver substance, many of them being lost during this stage. The writer's findings do not agree with those of Sinitsin, and in the description of this more conventional cycle, the long accepted nomenclature of the larval forms is mainly used. The observations recorded here are those made upon infested snails kept at a temperature varying slightly from 24° C. Upon dissection of the snail, several hours after miracidial attack, the sporocyst or sporocysts is, or are, found in various

positions in the snail tissues. Twelve hours after penetration this form appears as a sub-circular body, from 55-70μ in diameter. Many of the miracidial characteristics are retained, but all the epithelial cells and cilia have been discarded. The flame cells of the excretory system are unchanged in size, activity or relative position; the mouth and rudimentary intestine are retained, the head end being invaginated into the "body". The eye spot is intact or may be divided into two or more fragments. The germinal cells are similar in size and appearance to those in the miracidium. This twelve-hour stage is only faintly motile, and it is probable that movement is accomplished by means of the body movements of the snail.

The position of the twelve-hour sporocyst in the host tissues varies and, owing to its extremely small size, it is difficult to determine accurately. If the snail is exposed to a large number of miracidia the resultant sporocysts are found in many positions at the end of 12 hr. They have been found in the lymph spaces around the albuminiparous glands, but are generally inside or on the borders of the pulmonary sac. After a further period of development most of these early forms disappear and at the end of the fourth day a maximum of two per snail have been found intact, even in heavily infested specimens. It is probable that the sporocysts that develop normally do so in the pulmonary sac, and owing to the resistance of the host's tissues, only one or two are able to survive even in this favorable position.

The growth of the sporocyst is very slow for the first 48 hr., but on the third day a distinct difference in size is seen. The germ cells show the greatest growth, one being very much larger than the others. On the fourth day the outline of the head end of the primary miracidium is obscured, but the flame cells are still unchanged and the eye spot is still present and, in some specimens, intact. At this stage an uncertainty existed regarding the mode of escape of the next form; in one specimen a number of germinal cells were seen clinging to the outline of the sporocyst in the region of the posterior pore. This phenomenon was at first thought to be the "egg-laying" as described for *Fasciola* spp. by Sinitsin, but examination of all the other specimens in the lot and also many subsequent ones failed to reveal a similar case. It was found possible to produce this phenomenon by squeezing the sporocyst and it is fairly certain that the specimen under discussion had been injured during dissection of the snail tissues.

On or about the sixth day after miracidial attack, the largest germinal mass has developed a distinct mouth and at this time makes its escape from the sporocyst. In most cases the rest of the germinal masses also emerge simultaneously, although they are comparatively poorly developed and do not show distinct mouth parts. These forms have been constantly traced by daily examinations of one or more specimens in each lot of infested snails, and have always developed into mother rediae. The existence of another generation of sporocysts has never been observed in either of the snail hosts in Canada. The premature emergence of the germinal masses seems to be the usual procedure. The mother rediae are thus found in very moderate

numbers in the liver tissue of the snail host, each one present being in a slightly different stage of development. In one lot of *F. parva* that were exposed to one miracidium per snail, and which were examined on the twelfth day, one was found to contain a single immature mother redia and no other forms. Another specimen contained two rediae and no other forms. Still another specimen contained two rediae approximately 0.200 mm. in length and ten smaller ones in which the mouth parts and enterons were indistinct. Measurements of three lots of mother rediae which were emerging from the sporocysts showed the largest to be 0.150 mm. by 0.05 mm. and the smallest 0.09 mm. by 0.06 mm.

The development of the mother rediae continues and between the twentieth and thirtieth day the daughter rediae are well formed.

A reliable difference noted between the mother and daughter rediae is the retention of the strong anterior collar in the former generation. In daughter rediae containing cercariae no indication of the collar can be seen. This indicates that the collar is formed by a simple fold in the wall which is taken up by the stretching effect of cercarial growth.

Mother rediae containing well developed daughter rediae have not been found larger than 0.87 mm. by 0.21 mm. In *S. palustris nuttalliana* the development is retarded and the rediae are very much smaller. Unfortunately the birth pore in this form has not been clearly seen, but it is apparently situated immediately caudad of the anterior collar. The excretory system of the mother redia is complicated and has not been worked out; the flame cells are numerous in the mid-body and the excretory pore is easily seen at the caudal extremity.

Daughter Rediae

When the daughter rediae emerge from their mother, they have well developed mouth parts and large enterons, the latter often reaching to the junction of the middle and posterior thirds of the body length. The germinal mass rapidly splits into eight to twelve round bodies which form the embryo cercariae. The daughter rediae grow rapidly from a size of approximately 0.3 mm. by 0.1 mm. to a maximum size of 3 mm. long by 0.33 mm. in maximum width.

In *F. parva* which appears to be a very suitable snail host for this parasite, no more than ten embryo cercariae have been found developing in a single redia. Under favorable conditions all the cercariae in a redia develop rapidly and a short time before emerging are in a similar stage of development. The continual production of daughter rediae by the successive development of the mothers ensures the emergence of cercariae over a long period.

The mature daughter redia is thus a large form. It does not retain the anterior collar and has smaller locomotor appendages and thinner walls than the former generation. The enteron is retained and contains a varying amount of ingesta depending upon the stage of development of the cercariae; this organ is frequently empty after the cercariae have emerged. This generation is only feebly motile throughout its life.

In the other snail host in Canada (*S. palustris nuttalliana*), a peculiar variation in the production ot cercariae takes place. Instead of concurrent development of the embryos as in *F. parva*, they develop and mature successively. No more than three cercariae ready to emerge have been seen at one

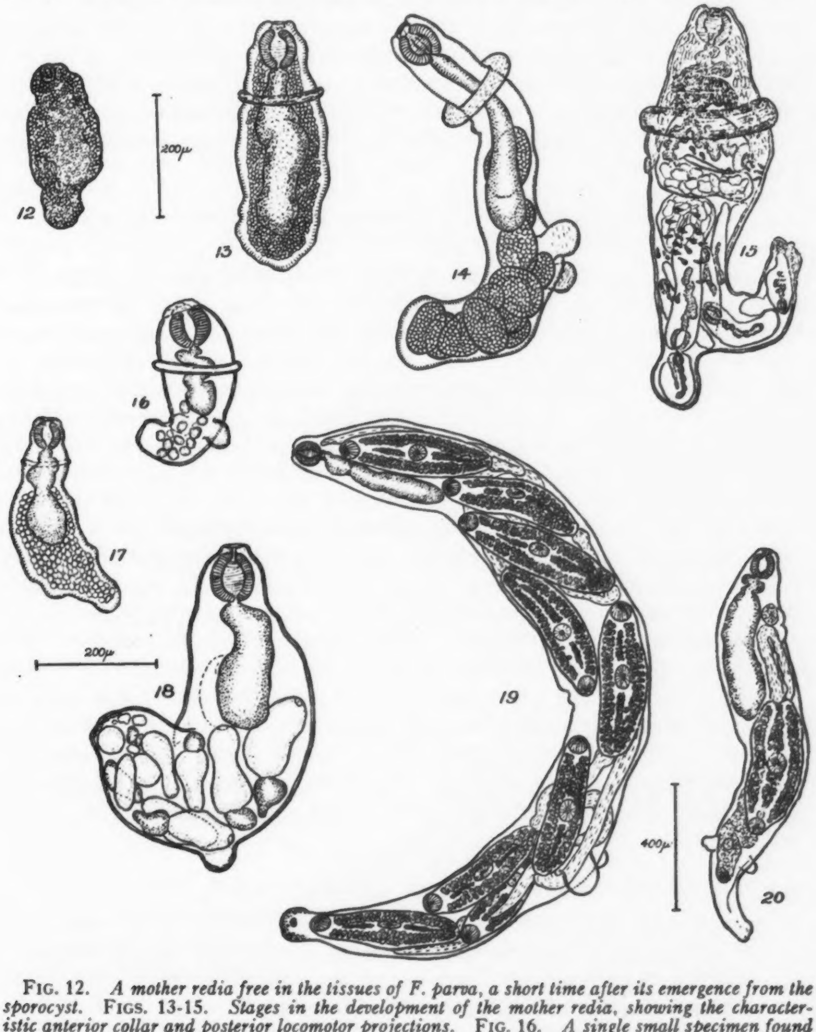

FIG. 12. *A mother redia free in the tissues of F. parva, a short time after its emergence from the sporocyst.* FIGS. 13-15. *Stages in the development of the mother redia, showing the characteristic anterior collar and posterior locomotor projections.* FIG. 16. *A single small specimen found in the liver of S. palustris nuttalliana on the fourteenth day of infestation.* FIG. 17. *An immature daughter redia shortly after emerging from a mother redia.* FIG. 18. *A typical daughter redia in the liver of F. parva, showing what has been found in this work to be a characteristic number of embryo cercariae.* FIG. 19. *An almost mature daughter redia in the liver of F. parva showing the concurrent development of cercariae. Compare this typical specimen with Fig. 20, which is the same generation in Stagnicola palustris nuttalliana.* FIG. 20. *A daughter redia from which most of the cercariae have emerged. This is typical of this second parthenogenetic generation in which the cercariae have developed successively*

time in a single redia. This finding has been constant throughout this work and appears to indicate that this snail is not a very suitable host. In order to show that this snail can and does act as a host throughout the intramolluscan cycle of the parasite, in spite of the fact that development is slower than in other snails, special observations were made upon the production of daughter rediae and cercariae. Results show that the numbers of daughter rediae do not vary from those found in the other species of snail. However, the production of cercariae is undoubtedly very much slower. This feature under natural conditions is consistent with the ecological finding that *S. palustris nuttalliana* lives under favorable conditions for longer periods than the other snail.

Experiment to Determine the Number of Daughter Rediae Produced Parthenogenetically from a Single Miracidium

Seven *S. palustris nuttalliana*, approximately ten days old, were each exposed to infestation by a single miracidium. They were then removed to a small tank to which was added clean vegetation but no extra source of calcium. These specimens retained the transparency of their shells and, by observation under low power magnification on the eleventh day, two were found to be infested. One specimen was killed on the twelfth day and five mother rediae were recovered. The other specimen was placed in a separate receptacle and observations upon the development of the parthenitae were continued. On the forty-ninth day several cercariae were seen to have emerged from the daughter rediae and to be moving rapidly in the badly damaged digestive gland of the snail. Many cercariae emerged on the fifty-third day and in two days 52 had encysted on the glass sides of the receptacle. The snail was then removed from the shell, and the digestive gland and adjacent tissues were fixed under a cover slip in warm 70% alcohol. The alcohol was then slowly replaced by glycerine, and the specimen was easily studied in detail. The forms present comprised a total of 21 large daughter rediae discharging cercariae, 16 small daughter redia containing an average of nine germinal masses and seven free cercariae in various stages of final development. To these must be added the 52 metacercariae obtained previously. The intramolluscan stages with their measurements are as follows:—

Large Daughter Rediae

(1) 1.33 mm. long by 0.211 mm. in maximum width. It contained one well developed cercaria 0.422 mm. in length, one partly developed, 0.165 mm. long, and two germinal masses approximately 50μ in diameter. Enteron 0.40 mm. long, oral sucker 72μ by 65μ in size.

(2) 1.05 mm. by 0.210 mm. Two cercariae, 0.351 mm. and 0.258 mm. in length. Enteron 0.390 mm. long, oral sucker 62μ in diameter.

(3) 1.17 mm. by 0.266 mm. Four cercariae, 0.446 mm., 0.435 mm., 0.280 mm. and 0.240 mm. in length. No germinal masses. Enteron 0.380 mm., oral sucker 60μ in diameter.

(4) 0.870 mm. by 0.203 mm. One cercaria 0.430 mm. long. Two germinal masses. Enteron 0.390 mm., oral sucker 60μ in diameter.

(5) 0.860 mm. by 0.195 mm. One cercaria 0.350 mm. long. Two large germinal masses. Enteron 0.395 mm.

(6) 0.664 mm. by 0.156 mm. One cercaria 0.275 mm. long. Seven germinal masses.

(7) 1.132 mm. by 0.234 mm. Cercariae 0.453 mm. by 0.172 mm. and 0.390 mm. by 0.125 mm. Germinal masses 0.177 mm. by 0.09 mm. and 0.082 mm. in diameter.

(8) 0.781 mm. by 0.150 mm. Enteron 0.318 mm. long. One cercaria 0.201 mm. Eight germinal masses.

(9) 0.790 mm. by 0.180 mm. Five cercariae, 0.273 mm., 0.203 mm., 0.160 mm., 0.160 mm. and 0.120 mm. Two small germinal masses.

(10) 0.870 mm. by 0.172 mm. Two cercariae 0.320 mm. and 0.340 mm.

(11) 0.780 mm. by 0.160 mm. One cercaria 0.205 mm. Three germinal masses.

(12) 1.05 mm. by 0.219 mm. Six cercariae 0.390 mm., 0.380 mm., 0.286 mm., 0.206 mm., 0.168 mm., and 0.160 mm. One germinal mass.

(13) 1.06 mm. by 0.250 mm. Three cercariae, 0.390 mm., 0.332 mm., and 0.312 mm.

(14) 1.00 mm. by 0.157 mm. One cercaria 0.320 mm.

(15) 0.890 mm. by 0.187 mm. Three cercariae 0.312 mm., 0.273 mm., and 0.250 mm. One small germinal mass.

(16) 1.18 mm. and 0.234 mm. One cercaria 0.280 mm. by 0.150 mm.

(17) 0.872 mm. by 0.158 mm. Two cercariae 0.370 mm. and 0.195 mm.

(18) 1.41 mm. by 0.235 mm. Six cercariae 0.400 mm., 0.397 mm., 0.395 mm., 0.375 mm., 0.203 mm., and 0.178 mm.

(19) 0.810 mm. by 0.195 mm. One cercaria 0.340 mm. Germinal masses ?

(20) 0.894 mm. by 0.155 mm. Two cercariae 0.235 mm., and 0.195 mm. Four germinal masses.

(21) 0.750 mm. by 0.162 mm. One cercaria 0.273 mm. Three (?) germinal masses.

In the above-mentioned rediae the enteron ranged in length from 0.318 mm. to 0.560 mm. The oral suckers had an average diameter of 60μ, the maximum size being 72μ by 62μ and the minimum 58μ in diameter.

Small Daughter Rediae containing cercarial germinal masses, the mean number of which was nine.

(1) 0.234 by 0.062 mm.
(2) 0.238 by 0.062 mm.
(3) 0.375 by 0.060 mm.
(4) 0.390 by 0.062 mm.
(5) 0.547 by 0.125 mm.
(6) 0.320 by 0.093 mm.
(7) 0.392 by 0.078 mm.
(8) 0.391 by 0.070 mm.
(9) 0.210 by 0.062 mm.
(10) 0.240 by 0.100 mm.
(11) 0.360 by 0.075 mm.
(12) 0.390 by 0.079 mm.
(13) 0.325 by 0.064 mm.
(14) 0.312 by 0.130 mm.
(15) 0.484 by 0.060 mm.
(16) 0.468 by 0.063 mm.

In the above-mentioned rediae the enteron varied greatly in size, the minimum length being 0.098 mm., and the maximum (No. 5) 0.290 mm. The oral suckers ranged in size from 56μ by 52μ to 45μ by 38μ.

In numbers, the above-mentioned daughter rediae do not differ from those found in *F. parva*. The more mature daughter rediae are, however, very much smaller owing to the fact that their cercariae have not developed at an equal rate. (Fig. 20.)

Cercariae

Of the nine cercariae present in the snail tissues, two encysted almost completely before they were fixed and two more only partly encysted owing to their immaturity. One which was completely, and two which were almost completely, coated with cystogenous material were successfully fixed and two immature ones were also fixed successfully.

The following measurements were taken:—

(1) Mature cercaria (capable of encysting). Body 0.386 mm. by 0.225 mm. Tail 0.910 mm. long by 0.064 mm. in median width.

(2) Immature cercaria. Body 0.296 mm. by 0.168 mm. Tail 0.880 mm. in length.

(3) Immature cercaria. Body 0.377 mm. by 0.202 mm. Tail curled.

(4) Completely encysted. Cyst 0.260 mm. by 0.220 mm. Tail 0.800 mm.

(5) Completely encysted. Cyst 0.255 mm. in diameter. Tail 0.937 mm.

(6) Almost mature. 0.317 mm. by 0.227 mm. Tail 0.590 mm. long.

(7) Almost mature. 0.305 mm. by 0.244 mm. Tail 0.670 mm. long.

The findings in this snail are consistent with observations made upon previous specimens of the same species which were not studied in detail, the parthenitae being used for individual study and dissection.

The Cercaria

The cercaria of *F. magna* is very similar in outward appearance and structure to that of other fasciolinid cercariae. The mean measurements of mature specimens, fixed in hot 70% alcohol, in a natural position are as follows:—

Body	0.300 – 0.321 mm. by 0.190 - 0.244 mm.
Tail	0.755 – 0.990 mm. by 0.045 - 0.057 mm.
Oral sucker	0.047 – 0.058 mm. in diameter (equat.).
Acetabulum	0.055 – 0.062 mm. in diameter.

Immature cercariae leave the daughter rediae as soon as the cystogenous glands are massed in the lateral fields (Pl. III, Fig. 5). At this stage the glands present the characteristic appearance described by Barlow as "the lower surface of a horse's hoof with the frog showing." It is this stage that has so often been taken from the snail's tissues and described as mature cercariae. Bovien (1931) pointed out this error in his study of *Cercaria Fasciolae hepaticae*.

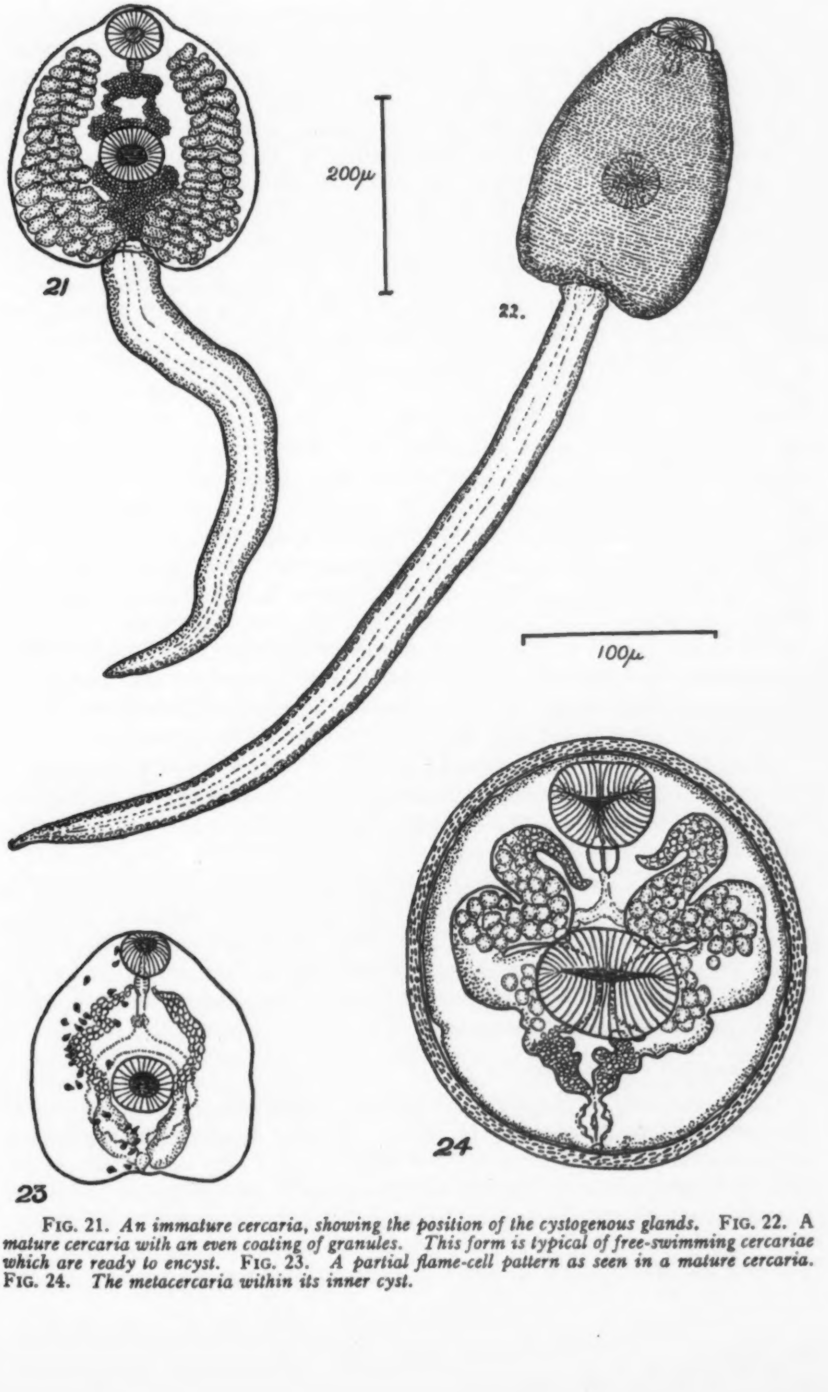

FIG. 21. *An immature cercaria, showing the position of the cystogenous glands.* FIG. 22. *A mature cercaria with an even coating of granules. This form is typical of free-swimming cercariae which are ready to encyst.* FIG. 23. *A partial flame-cell pattern as seen in a mature cercaria.* FIG. 24. *The metacercaria within its inner cyst.*

The cercariae of *F. magna* remain in the digestive gland of the snail for at least four days before emerging. When they do emerge they are mature, ready to encyst, and present a striking difference in appearance to those in the snail. They have acquired an even coating of cystogenous granules arranged in definite rows and which completely encircle the body: from 110 to 125 of these rows have been counted in individual specimens. The "hoof" appearance of the glands has been dissipated and the acetabulum is only seen as a faint outline under the coating. The granular coating is from 11μ to 13μ in thickness and is somewhat denser over the anterior part of the body where the rows are closer together (Pl. III, Fig. 6, and Fig. 22.)

The anterior extremity is the only part of the body which projects from this coating, and shows the characteristic spiny cuticula. Owing to the opacity of the granules the excretory system and the intestinal tract cannot be seen in mature specimens and a modified technique is necessary to remove the coating. This was done by pipetting the cercariae, as they emerged, into egg albumen, a cover slip then being closely applied. As they commenced to encyst, the slip was moved at approximately one-minute intervals until all the cystogenous material was removed. The specimen could then be retained in a live state in the medium for several hours.

The intestinal tract is essentially the same as that of *Fasciola* spp., the caeca being somewhat spiralled. Unfortunately it has not been possible to discern the entire solenocyte system of the excretory apparatus. However, sufficient flame cells were seen in one specimen (Fig. 23) to indicate that the formula conforms with that of the fundamental flame-cell pattern of the group (Faust, 1924). Minute spines are present on the anterior part of the body but do not appear to be present caudad of the acetabulum.

The tail is attached on the ventral surface at a point anterior to the posterior end of the body. The point of attachment in mature specimens is obscured by the granular coating. This organ is strongly muscular and is relatively larger than any other in the group.

In obtaining mature cercariae for study natural emergence was awaited and the specimens were immediately fixed in hot 70% alcohol. According to measurements of live specimens, this technique did not cause undue shrinkage. They were later dehydrated, cleared and then mounted in balsam. Staining was useless owing to the thickness of the granular coating which could not be satisfactorily cleared.

The Emergence of Cercariae

During the early stages of the infestation experiments, it was noted that all the cercariae emerged during the night. In order to check the consistency of this nocturnal phenomenon several infested snails were kept under observation all night. During the first night four snails were observed which had already produced many cercariae during previous nights and were apparently heavily infested. Two of these produced no cercariae. In one snail, nine emerged between 11:00 p.m. and 11:15 p.m., two being the greatest number

to appear simultaneously. Three cercariae emerged together from the other snail at 11:05 p.m. During the next night four more snails were observed but only one produced cercariae, three appearing between 1:00 a.m. and 1:30 a.m. Nine snails were observed at hourly intervals during another night, and forty cercariae appeared between 1:00 a.m. and 2:00 a.m., and five more during the next hour.

The above investigations were conducted under a weak artificial light, the snails being placed in individual petri dishes during the first two evenings and in one large one during the third night. In order to check this nocturnal habit further, 24 infested snails were placed in petri dishes and the encysted cercariae were counted at 5:00 p.m. and 8:30 a.m. for two days and nights. All the cercariae emerged at night between these hours. However, in one case that was not kept under observation a colleague reported the diurnal emergence of two cercariae, between 1:00 p.m. and 5:00 p.m. In two other cases the great activity of the tails which were still attached to the cysts at 8:30 a.m. indicated that the emergence had been more recent than six hours, although activity frequently lasts for three hours. An attempt was made to induce cercariae to emerge diurnally. All sources of light were removed from six dishes containing 12 snails during the daytime and a strong artificial light was applied at night. This was continued for three days and nights but in each case only nocturnal emergence occurred.

Depths at which Cercariae Encyst

Eight snails (*F. parva*) which commenced to discharge cercariae on the fifty-sixth day were placed in a standard small aquarium jar in 15 cm. of water. Vegetation was removed with the exception of a few pieces of grass and a piece of lettuce. One side of the jar was directed towards a window, two sides were covered with cardboard and one was exposed to dull light in the room. The sources of light were street lamps outside of the building and were of low intensity. Subsequent examination did not show a preference for the exposed sides, the greatest number of cercariae having encysted on a covered side adjacent to the food supply of the snails. At the end of 48 hr., 1004 encysted metacercariae were counted on the glass sides at the following depths below the surface of the water:—

1st centimetre	314	7th centimetre	22
2nd centimetre	316	8th centimetre	3
3rd centimetre	158	9th centimetre	4
4th centimetre	89	10th centimetre	1
5th centimetre	60	Below	0
6th centimetre	37	On the bottom	0

In a "moist-chamber" dish, in which were kept several snails of the same species as before, cysts were counted at the following depths:—

0 – 1 cm.—156 cysts.	2 – 3 cm.—140 cysts.
1 – 2 cm.—200 cysts.	3 – 4 cm.— 95 cysts.

On an area of the bottom, equal to the area of the wall upon which the above were counted, were 14 cysts. No cyst has ever been observed on the surface of the water, but they have been frequently found on the outer surface of the shell of the snail host. Although there was an abundance of sedge leaves, grass, algae and some lettuce in this and one other dish in which many cercariae emerged, all of them encysted on the glass. In order to obtain encysted forms on vegetation (Pl. III, Fig. 7), it was necessary to cover completely the inner surface of the glass sides of a vessel. Lettuce leaves were used for this purpose.

These findings indicate that the encysting habits are peculiar in that they are almost always nocturnal and that the cercariae do not encyst as readily on grass as do *Cercariae Fasciolae hepaticae*. This, and the complete absence of cysts on the surface of the water, show that infestation of the final host does not take place through drinking but rather by the ingestion of coarse vegetation.

The Metacercaria

The outer cyst of the metacercaria of *F. magna* is sub-circular in outline, 0.248 mm. to 0.275 mm. in diameter. These measurements do not include the adhesive substance around the cyst which projects from the outline for a distance of 12μ to 40μ. From a lateral view, the cysts are helmet-shaped, from 0.120 mm. to 0.130 mm. high. They are of a dark gold color when fresh, but with age become light yellow. The surface is coarsely reticular over the dome, but the side in contact with the object upon which they have encysted is smooth and the wall composed of a thin transparent adhesive substance containing few cells. The inner cyst is somewhat discus-shaped, the ventral surface concave to a depth of 30μ, and the dorsal surface convex to a similar degree. The measurements are, diameter 0.214 to 0.235 mm., maximum thickness 90 to 95μ. The hard inner cyst wall is 5 to 7μ in thickness, smooth in outline and in close contact with the metacercaria. The larva cannot be dissected intact from this cyst. The encysted metacercariae are unable to infest the definitive host if their outer cysts are removed. This was demonstrated by feeding twenty denuded inner cysts to each of two rabbits. As a control two other rabbits were fed with whole cysts at the same time. Post-mortem examinations on the twenty-eighth day revealed that the denuded inner cysts had not caused an infestation, but in each of the other rabbits, one marita was found. Owing to the findings of other workers, that metacercariae of related trematodes are unable to withstand digestion without an intact outer cyst, this fact was accepted without further investigation.

The oral sucker, acetabulum and excretory ducts conform to the general outline of related species. The excretory granules are coarse, and obscure the outline of the intestinal caeca.

Resistance of the Metacercaria to Partial Desiccation

The normally encysted metacercariae are very resistant to partial desiccation. One experimental jar, used previously for the investigation upon the depths of encysting, was retained for observations upon the effect of desiccation. The vegetation was removed and the water was poured off to a depth of 5 cm. All the encysted cercariae were then above the water line and the jar was left in the aquarium room at a mean temperature of 24° C. At bi-weekly intervals several metacercariae were removed and examined and at the end of sixty days, only a small percentage had been destroyed. At this time a rabbit was successfully infested with a marita from these metacercariae. On the ninety-second day the jar was removed to another room and at the end of 24 hr. all the larvae were found to be destroyed by desiccation.

At the time of removal from the aquarium room only half of the water below the cysts had evaporated, thus indicating a high relative humidity in this room. The relative humidity of the second room was then recorded and found to be 20%, this low degree being brought about by the steam heating appliances.

From this observation, it is apparent that the larva is not destroyed by desiccation while a certain degree of humidity is present in the atmosphere. It is generally agreed that the metacercarial stage of fasciolinid trematoda is readily destroyed by desiccation, and it has hitherto been believed that the larvae must remain in very damp surroundings if they are to survive. However, in spite of the fact that sheep have been experimentally infested by feeding "cured" hay, no attempt has previously been made to estimate the degree of relative humidity at which the larvae are destroyed. In order to clarify this point, a set of containers was prepared in which the relative humidity was controlled over solutions of sulphuric acid. The surfaces of the acid solutions were broken by coarse washed and baked sand, and the metacercariae, on glass, were suspended in the jars above the solutions. In order to eliminate any danger of injury to cysts, an aquarium jar which contained many newly encysted larvae was cut up into suitable pieces which were not allowed to become dry. The pieces of glass upon which numerous cercariae were encysted were placed in the test chambers immediately after the excess moisture was removed and the surfaces appeared to be dry. The cysts on some pieces were immediately examined in order to control this method of preliminary drying and all were found to be alive. In determining whether the larvae were alive or dead, the outer cyst was carefully dissected away and the outline of the inner cyst then examined. If this was normal in appearance and if the excretory tubules of the larva were in place and the

flame cells were active, it was considered to be alive. Dead metacercariae were easily identified by their partially collapsed inner cyst, the displaced excretory tubules and an absence of movement. The results of this biophysical experiment are shown in Table I.

TABLE I

RELATIVE HUMIDITY TESTS

Relative humidity, %	Hours tested	No. of cysts examined	No. of cysts dead	No. of cysts alive
15	24	20	20	0
15	65	26	26	0
20	24	11	7	4
20	65	39	39	0
25	24	25	24	1
25	65	36	35	1 (?)
30	24	10	5	5
30	65	35	34	1 (?)
40	24	12	9	3
40	65	37	31	6
50	24	11	4	7
50	65	40	31	9
60	24	18	14	4
60	62	33	28	5
70	24	14	3	11
70	65	19	11	8
70	120	16	15	1
80	24	10	1	9
80	65	35	25	10
80	120	20	15	5
90	120	17	4	13
95	120	30	0	30
95	240	20	0	20
95	480	22	2	20
95	576	10	1	9

Lack of suitable material prevented an extension of this investigation, but the results in Table I indicate that a very high humidity is necessary to ensure survival of metacercariae encysted on a non-porous substance and exposed to air. However, those that encyst on vegetation may survive very much longer in air even at low relative humidities. The comparatively large amount of water retained by plants could probably be utilized to prevent lethal desiccation of the cysts.

In summarizing the above observations on the effect of drying on metacercariae it is apparent that they possess individual powers of resistance. It is probable that they are able to exist under natural conditions for a considerable time after the water under which they encysted has receded. Practical tests on hay from an endemic area are now being conducted and it is hoped that the results will demonstrate the advisability of drying the hay for longer periods in order to ensure a greater death rate of the encysted metacercariae. Rajcevic (1929) has shown that metacercariae of *F. hepatica* remained alive in stacked hay for 17 months (two summers and one winter), proving the viability by feeding tests on sheep. Hay, therefore, must be recognized as a probable source of infestations by *F. magna* as well as by *F. hepatica*.

Artificial Infestations in Rabbits, Sheep and Guinea Pigs

In the experiments summarized in Table II all the animals used were young healthy stock, born and raised in this Institute. The animals used for other experiments, and subsequently killed and examined, were considered as controls:

TABLE II

INFESTATION EXPERIMENTS

Hosts	Cysts	Period of encystment	Period, feeding-slaughter, days	No. of maritae	Size of maritae, mm.
Rabbits Nos.					
1	10	3 days	33	0	— (Peritoneal lesions present)
2	10	12 hr.	103	1	7×4
3	9	8 hr. (or less)	33	2	3.7×1.8
4	10	12-24 hr.	30	1	2.0×1.2 (in peritoneal cavity)
5	10	12-24 hr.	28	1	1.2×1.1
6	15	4-5 days	28	1	3×1
7	15	4-5 days	28	0	—
*Sheep					
1	15	2-3 days	52	1	5.5×2.7
2	75	4 days	Still living, no eggs at end of 77 days		
3	75	4-6 days	Still living, no eggs at end of 77 days		
4	72	4-6 days	Still living, no eggs at end of 77 days		
Guinea pigs					
1	10	4-5 days	41	0	—
2	25	30-31 days	63	1	8.8×4.5

* *See page 214. "Further notes on Ovis aries as a definitive host of F. magna."*

These records do not include those animals used for the preliminary viability tests of dried metacercariae. All the metacercariae used in the infestations were constantly under the surface of water until used, when they were administered in a gelatine capsule filled with bran and ground oats. In the case of the rabbits the capsules were broken and the contents chewed before being finally swallowed. It is improbable in these cases that all the metacercariae arrived in the stomach intact in their outer cysts.

Owing to the extremely slow rate of development to the mature adult stage, it has not yet been determined whether maritae are able to develop to an egg-laying stage in the rabbit. Rabbits which received metacercariae were still negative to feces examinations for eggs four months later. One calf, repeatedly fed with viable metacercariae over a period of 20 days, was not passing eggs when the weekly examination was last made at the end of the twenty-second week.

Development of the Adult

As shown in Table II the young maritae developed very slowly in the animals which were artificially infested. In rabbits they wander over the peritoneum of the abdominal cavity for a long time. In one instance (No. 5) one was found under the peritoneum of the abdominal wall on the twenty-eighth day.

Sinuous caseated tracks mark the route taken by the parasite in the cavity. These tracks often lead to the surface of the liver into which the route is easily traced. The parasite wanders about in the liver tissue until finally encapsulated by fibrous tissue. In laboratory animals no complete encapsulation has so far been observed, probably owing to the limited period available before slaughter. In *Cervus canadensis* the lesion caused by young forms has been studied and in no case has the parasite been found in an egg-producing stage unless the encapsulation was complete. Forms have been found which have attained a size of 4 by 2 cm., which were in complete fibrous cavities and were still sexually immature.

Specimens removed from fresh liver and placed in normal saline at 38° C. are extremely active, but are not able to progress through the solution, their activity being confined to strong muscular contractions and bending processes. This muscular activity, combined with their cuticular spines as described by Stiles and Sinitsin, explains their ability to migrate through dense animal tissues with comparative ease.

The genital primordia are present in the maritae at a very early age, and have been clearly seen in all young specimens listed in Table II. However, further development is very slow and the period required to reach sexual maturity is still unknown.*

The largest adult recorded during this work was 8.1 cm. long by 3.2 cm. in greatest width. This measurement was made upon a live specimen stretched out on the palm of the hand. All anatomical details conform to the description of this trematode by Stiles, and any attempt to enlarge upon this thorough study would be superfluous.

The Location and Lesion Produced in the Tissues of the Definitive Host

Although *F. magna* is repeatedly mentioned as occurring in the lungs of its definitive hosts, it is unlikely that this is a common location.* The writer has never found this parasite in the lungs of heavily infested wapiti or any other ruminants or rodents, although the lung tissue was always examined with care. Francis (1891) states that he found them only in the liver of cattle in Texas. Curtice (1897) reported three cases of pulmonary distomatosis due to flukes which he later stated to be *F. magna* and also cites three more cases which were recorded in the "Veterinary Review", 1882, by A. J. Murray. I am unable to find any other definite records of pulmonary fascioloidiasis and am inclined to consider the occurrence as reported as an abnormal migration due to its occurrence in an abnormal host.

F. hepatica and *F. gigantica* are not infrequently found in the lungs of *Bos taurus*, in most cases being encysted and immature.

**See page 214.* *"Further notes on Ovis aries as a definitive host of F. magna."*

Francis described the migration of the marita in the liver tissue of *Bos taurus*. The channels that he observed in the tissue were large: "they admit the little finger and seem to heal or fill up soon after, leaving a red scar." He described the subsequent cyst, stating that the wall becomes dense and tough and is usually coated with a grit-like substance. He thought they died in these cysts, amidst the black fluid in which he found myriads of eggs. He found a maximum of five eggs in the entire quantity of bile that he collected from one animal, but states that he was not positive that those were eggs of *F. magna*. These findings in regard to the exodus of eggs into the bile system in *Bos taurus* agree with the recent findings of Sinitsin (1930 and 1933). It is peculiar that all the workers who have studied these eggs appear to have obtained their material from the fluke cysts in the liver of cattle and not from the feces. In the absence of adequate cattle data I have to rely on the observations of Francis and Sinitsin in propounding a hypothesis that egg evacuation in fascioloidiasis of cattle is abnormal and in some cases s nonexistent. In *Bos grunniens*, as mentioned elsewhere, this is true in at least one case.

In addition to the above data regarding the abnormal parasitic life of this trematode in *Bos* spp., are the observations made upon the peculiar black discoloration on the mesentery, peritoneum and in the lymph glands, which was described by Dinwiddie in *Bos taurus*, and which is constantly seen in *Bison bison* and on two occasions has been observed in *Bos grunniens*.

In *Cervus canadensis* this discoloration occurs only when a closed or nonfunctional fluke cyst is present in the liver (See Plate IV, Fig. 1). When normal cysts only are present, discoloration of the tissues other than slightly darkened lymph glands and occasional black specks throughout the liver tissue, has not been observed, although numerous specimens have been examined. In the case of *Bos grunniens*, when all the cysts were "closed" the tissue discoloration was excessive, the liver being dark in color and the peritoneum, lymph glands and mesentery all containing blackened areas. Unfortunately, the whole livers of only two *Odocoileus virginianus* have been examined; in both cases the infestation was light and the cysts were open and no pigmented areas were present.

Salomon (1932), reporting the record in *Cervus elaphus* in Germany, described the macroscopic appearance of the liver, stating that there appeared to be no tissue reaction beyond the confines of the distended bile ducts. In the annual report of the Conservation Department of New York State, U.S.A., for 1933, cases of *F. magna* infestation of the livers of *Odocoileus virginianus* are reported but no mention is made of undue tissue reaction. In conversation and correspondence with observers who have seen fascioloidiasis in deer, no observations of severe tissue reactions or undue discoloration have been reported. It therefore appears probable from these findings in Cervidae, that this parasite finds a normal host in members of the Cervidae and is able to continue its normal life, producing eggs which are evacuated into the alimentary canal through the bile ducts.

In Bovidae, however, it appears that the tissue reaction to the presence of the trematode is so great that the "fluke cyst" is completely closed off in the majority of cases from the bile duct system and the powers of the parasite to reproduce are thus destroyed.

In order to support this hypothesis the tissue reaction has been studied in three hosts which harbored flukes in lesions typical of their species. The first is *Odocoileus virginianus* which was infested with three large trematodes, measuring 8.2 by 2.8 cm., 7.2 by 3.4 cm., and 8.6 by 3.1 cm., when fixed under slight pressure; these were contained in a single cyst from which open ducts conveyed the eggs normally. The second is *Cervus canadensis*, which was infested with fifteen flukes. In this case the infestation was so heavy that one cyst was closed and consequently the hepatic lymph glands and liver tissue were pigmented. (Pl. IV, Fig. 1.) The third host is *Bison bison*, in which the liver was infested with four flukes in two cysts. Both cysts were closed and extensive pigmentation was present in the tissues adjacent to the liver. Hadwen (1916) states that in an infested liver of *Odocoileus columbianus* the ducts were found to be dilated into pockets, these containing more "inky" bile than those caused by *F. hepatica*. However, accompanying photographs show that these lesions of fascioloidiasis were normal cavities with comparatively little pigmentation, and support my findings in other Cervidae.

The descriptions of the lesions are made under the names "open cavity" and "true cyst". The former name implies a continued evacuation of eggs into the bile ducts and the latter indicates a lesion which prevents egg evacuation, by occlusion of intercepted bile ducts.

Histopathology of the Liver Lesion and its Bearing on the Epizoology of *Fascioloidiasis magna*

Open Cavities

In cross section the fibrous cavities present a striking difference in structure from the lesions of other diseases caused by parasites in tissue. The wall is composed of fairly loose layers of fibrous tissue, the older tissue being on the outer surface of the cavity in contact with the liver tissue. The layers are successively more recent, the inner surface being composed of a layer of fine collagen fibrils and fibroblasts. The fibrous wall includes a blood-vascular system, and small bile ducts penetrate into the cavity. The outer layer of the wall is thus sharply differentiated from the surrounding liver tissue and, except in the region of bile ducts, there is no intermingling of liver and fibrous tissue.

The ducts, which have been intercepted in their course by the formation of the cavity, are thus divided into afferent and efferent ducts for the benefit of the enclosed parasite. The former supply the cavity with bile and the latter carry away excess bile, vomitus of the trematode, and eggs. While even a single cross section of a normal cavity reveals several bile ducts actually entering the cavity, no blood vessels have been seen doing likewise. In all probability these vessels are merely diverted from their course and some of

them may take part in the newly organized blood-vascular system of the wall of the lesion. In normal open cavities the bile ducts continue to function in their new capacity and, except for a slight fibrosis of the wall of the efferent ducts, they appear to be unharmed. In older cavities containing large mature flukes, the efferent vessels are seen to be fibrosed to a greater extent, in some cases being completely occluded by excess fibrous tissue which surrounds a mass of black vomitus and eggs. In most cases however, evacuation of eggs and detritus by the bile system is continued for a considerable time by the other open ducts.

In sections of the lesions, the path of primary migration by the young marita can be seen as lines of fibrosis in the liver tissue which have no direct relation to the bile system. This observation agrees with the observations made by Francis upon the migration through the liver tissue of cattle and with my own observations upon the migration in laboratory animals. It is evident that the marita comes to rest in the liver tissue as soon as the host's tissue reaction enforces it, and the cavity is then formed. The young fibrous tissue which has followed the path of the migrating marita and which approximates the final location is included in the formation of the fibrous cavity. At first the fibrous wall is composed only of a few strands of fibrous tissue (Pl. V, Fig. 2), but the growth and activity of the parasite enlarges the cavity until a surrounding area containing small masses of fibrosis, blood vessels and bile ducts is included. The liver tissue is destroyed by pressure atrophy until some time after the cavity has reached its maximum size, when normal liver cells are again found adjoining the fibrous wall. The size of the cavity varies greatly according to the size of the parasite or parasites, or according to the number. The largest open cavity observed was roughly spherical in outline and 4 cm. in diameter and contained three trematodes, the maximum number seen. The fibrous wall varies between 1.00 and 1.75 mm. in thickness.

The number of efferent and afferent bile ducts varies according to the size of the cavity. The trematode or trematodes lie coiled in the cavity, the ventral surface nearly always being in contact with the fibrous wall; this position is probably adopted to enable the acetabulum to fulfil its function. A comparatively small number of eggs is present in the cavity; these are mingled with a small quantity of bile and vomitus which appears microscopically as a black amorphous mass. In some cases masses of eggs and vomitus pigment are present in the wall, suggesting a fairly rapid growth of fibrous tissue during an egg-producing stage of the trematode's life in the cavity.

True or Closed Cysts

The above description covers briefly the general appearance of nearly all mature fluke cavities in the Cervidae. However, in heavy infestations in these animals there is often at least one closed cavity, which is a true adventitious cyst, filled with black fluid composed of bile, eggs, vomitus and often shreds of decomposed trematodes. This type of cyst is the typical lesion of

fascioloidiasis in the larger Bovidae, and indicates the important difference in the host's tissue reaction. It is this type of lesion that has been from time to time described as the ordinary lesion caused by *F. magna* in cattle. However, all previous workers appear to have merely described the external appearance of the liver and the appearance of the contained fluid and the incised cyst. From a comparative study it soon became certain that the closed cyst containing black fluid was the result of an attack by the parasite which had been overcome by the host's defence reactions. A study of this type of lesion shows an all-important difference in structure and an almost total obliteration of the ducts so essential for the normal evacuation of the trematode's eggs. This structure, in *Bos taurus*, can be explained by the well known fact that the fibrous tissue reaction to foreign bodies is particularly well marked in this animal. As stated by Dévé (1920) ". . . . . on pourrait arguer que cette différence dans le mode de réaction des tissus au voisinage du parasite tient simplement à la différence du terrain, à l'espèce animale. Bien connue est, en effet, la tendance des tissue du bœuf à la réaction fibreuse et à la calcification des lésions parasitaires ou tuberculeuses."

The cyst is thick-walled, the fibrous tissue layers being more numerous and closer than those in the wall of a normal cavity. Both afferent and efferent bile ducts are totally occluded and are marked by tracts of fibrous tissue. The outline of the cyst is not well differentiated from the liver tissue owing to the extensive fibrosis of the bile ducts which involves the surrounding tissue. In some cases pigmentation is extensive, the pigment mass being very similar in microscopic appearance to the masses seen in cross sections of the caecal branches of the trematode. The chains of liver cells adjacent to the masses of fibrous tissue and pigmented areas are normal in appearance, and there is no evidence of recent inflammatory processes. No normal bile ducts have been found opening into a cyst which was filled with black bile and vomitus, and I am convinced that for only a short period during the trematode's early life in large Bovidae could they be found. In *Bos grunniens*, immature specimens of *F. magna* were found in closed cysts surrounded by the fluid which, of course, contained no eggs. It is probable that the trematode rarely reaches sexual maturity before being completely enclosed. That it does accomplish this sometimes is evidenced by the recording of a few eggs in the feces of *Bos taurus* by Sinitsin and the record of up to five eggs in the gall bladder of this host by Francis.

In addition to data obtained from past records in *Bos taurus* and the present examinations of a limited number of liver specimens from *Bison bison* and *Bos grunniens*, I am able to quote the work of Dr. R. Waddy, Inspector in the Health of Animals Branch. Dr. Waddy has made post-mortem examinations of some thousands of *Bison bison* and a few hybrids (domestic × bison) and mentions in his reports that extensive pigmentation occurs in the lymph glands and tissues adjacent to the liver in infested animals. This indicates a true cystic formation of the lesion. These reports also state that not more than four flukes have been found in one animal.

It is, therefore, apparent that the larger Bovidae, owing to their power of tissue regeneration, harbor the adult trematodes in true adventitious cysts. These animals are, therefore, unsuitable hosts for *F. magna*, preventing the completion of its normal processes of reproduction. It is altogether unlikely that the animals in which the efferent ducts are not completely closed can evacuate sufficient trematode eggs in their feces to offset the great hazards in the life cycle of the parasite. In support of this hypothesis one must consider that 1.85 eggs per gm. of feces are evacuated continually by a typical *Cervus* sp., infested with one trematode. This, in *Cervus canadensis*, means that each adult *F. magna* is producing at least six thousand eggs per day, all of which are evacuated normally. Even so, we do not encounter heavy infestations in *Cervus* spp. in most parts of North America, in spite of the known fact that several common snails can act as intermediate hosts. In cases of areas abnormally crowded with definitive hosts and rich in suitable snail life, we do see instances of super-infestations. I have examined an area, adjacent to the endemic locality to which reference has been made, that harbored similar snail life and over which a large herd of cattle ranged. There is no evidence of Cervidae and no case of fascioloidiasis has been observed during the past three years. On another large enclosed area only 13 miles from the above-mentioned ranch, in which several *Odocoileus hemionus* are enclosed, fascioloidiasis was present in the hybrid *B. taurus* × *B. bison*. Cervidae range freely over the area in which the Canadian bison are enclosed. The light infestations in these Bovidae can only be explained by reference to their feeding habits. Bison do not feed readily in low areas, visiting them only for the purpose of drinking, and retiring to higher land to obtain the preferred "prairie wool," to which cercariae would have no access.

It seems, therefore, very unlikely that *Bos taurus* and related Bovidae will ever be severely infested with *F. magna* unless Cervidae have access to their grazing lands. In other words, fascioloidiasis in large Bovidae occurs probably only in the presence of Cervidae. Unfortunately, in arriving at the above-mentioned conclusion the part played by the smaller Bovidae (*i.e.*, *Ovis aries*) in propagating this disease cannot be stated.* However, experiments are in progress in an attempt to ascertain this point, but owing to the weaker power of tissue regeneration in *Ovis aries* than in *Bos taurus*, it is more than possible that these animals may play a role similar to the Cervidae. Sinitsin's hypothesis that sheep are not commonly infested, because *F. magna* can only complete its life cycle in areas in which there is an abundance of water for at least four months, is not supported by the present work.

Control of Fascioloidiasis Magna

The control of this parasitic disease differs in several respects from the measures employed for fascioliasis hepatica. Anthelmintic medication would probably be entirely ineffective owing to the location of the parasite in the

**See page 214.* *"Further notes on Ovis aries as a definitive host of F. magna."*

tissue of the host. Prophylactic measures must be employed following careful consideration of the definitive host involved and the character of the endemic area.

If the health of *Bos taurus* or other large Bovidae is to be protected, then all Cervidae should be eliminated from the grazing lands. This measure should reduce the danger of harmful infestations to a minimum. Care should be taken in importing Cervidae from North America, Italy or Germany. Such animals should not have access to areas inhabited by other Cervidae or Bovidae until examinations of feces have failed to reveal ova of *F. magna* for at least five months. As stated previously, it is unlikely that the importation of cattle from an infested area will cause an outbreak of fascioloidiasis in Bovidae in a previously free area, unless Cervidae are present.

In parks and other areas where deer are kept, or where there is an intermingling of these animals with Bovidae, destruction of the intermediate hosts is necessary. Much has been written about methods of snail destruction and copper sulphate is now in general use as the lethal agent. However, in the disease caused by *F. magna*, consideration must be given to the fact that three genera of Lymnaeidae are involved in the life cycle. One snail, *S. palustris nuttalliana*, shown in this paper to be an intermediate host in Canada, is ecologically very dissimilar to the other groups. This species inhabits permanent or semi-permanent, warm, stagnant bodies of water which are not favorable habitats for any other snails involved in the life cycles of liver flukes of ruminants. *Fossaria* spp. on the other hand are fairly consistent in their habitats, and methods so often described for snail destruction will be effective.

In Alberta, Canada, the area described under the heading of "Snail Hosts with Notes on their Ecology" was first treated, using copper sulphate for snail destruction. The stagnant bodies of water which harbored *S. palustris nuttalliana* were considered and a rough estimate was taken of the volume of water present. One part, by weight, of powdered copper sulphate was mixed with five parts of sand. After weighing but before mixing, the sand was slightly dampened to prevent undue blowing of the copper sulphate. This mixture was broadcast by hand over the swamps in amounts varying with the depth of surface water present. In swamps in which there was an average depth of water of 18 in., the mixture was applied at the rate of approximately 500 lb. per acre. This produced approximately a 1 in 430,000 solution in the swamps, which is theoretically sufficient to produce the desired lethal effect upon the snails, and to allow for loss by fixation of the salt by organic matter. In 1933 this method was completely successful in destroying this snail in several swamps in Alberta, and in May, 1934, no snails could be found in the treated waters.

One other method was tried in an attempt to destroy snails under the climatic conditions encountered in autumn on some Alberta prairie lands. Two swamps, which were known habitats for great numbers of *S. palustris nuttalliana* were selected. One was treated with the copper sulphate and sand

mixture at a rate of 250 lb. per acre when both were completely dry in early October. These swamps were dry until filled with snow water during the following spring. In May, 1934, both swamps were carefully examined and in the treated one only two young specimens of the snail could be found. In the untreated swamp, innumerable adult and juvenile specimens of the snail were present. This experiment, while being in no way conclusive, indicates a possible simple method of applying the lethal agent in western Canada.

In destroying *F. parva*, the above-mentioned mixture was broadcast over the described low, damp areas in May, when some free water was still present. The mixture was broadcast at the rate of approximately 250 lb. per acre, special attention being paid to water-filled animal tracks. Examinations made one week later did not reveal any living snails. It is probable that this method, or the one tried in October for destroying *S. palustris nuttalliana*, will be entirely satisfactory.

In recommending the destruction of *S. palustris nuttalliana* and *Pseudosuccinea columella* by applications of copper sulphate, special attention must be given to the fact that these snails, and closely related *Stagnicola* spp., frequently live in water inhabited by fish which may be killed by this salt. In these cases it would be advisable to rely upon the segregation of ruminants.

Acknowledgments

It is with great pleasure that I acknowledge the help and co-operation extended to me by the National Parks Branch, Department of the Interior, Ottawa. Mr. J. B. Harkin, Commissioner of Parks, and Mr. Hoyes Lloyd, Chief of the Wild Life Division, with whose support this work was accomplished, have at all times extended the utmost co-operation and support on behalf of their Branch. Mr. A. G. Smith, Superintendent of Wainwright Park, provided excellent camping accommodation, help and equipment, and has at all times made my work pleasant and successful by his unselfish interest. His staff has, from time to time collected material, kept records and employed control measures, all of which work has been invaluable. The efficient methods employed by the Branch in preserving the health of their animals cannot be regarded too highly and augur well for the future of Canadian wild life and also domestic stock.

I also wish to express great appreciation of the co-operation of Mr. A. LaRocque of the Geological Survey, Department of Mines, and Dr. F. C. Baker, University of Illinois, who have so kindly identified the snails and have provided me with valuable records and much helpful advice.

Dr. E. A. Watson, Chief of the Pathological Division of the Health of Animals Branch, Department of Agriculture, under whom the preliminary survey work was commenced, kindly consented to the proposal that the work should be continued under this Institute, and I acknowledge his kind co-operation.

Further Notes on *Ovis aries* as a Definitive Host of *F. magna.*

Since the manuscript of this paper was submitted to the publishers, certain developments in the continued experiments on infestation of sheep have been noted. It is deemed necessary to include herewith some short notes on the development of the trematode in sheep and on its effect upon the host tissues.

Sheep No. 2 died on the 149th day after the ingestion of 75 encysted metacercariae. Although this animal was only parasitized by seven trematodes the liver tissue was severely damaged, and the animal exhibited marked emaciation. The largest trematode present was 3.2 cm. long by 1.9 cm. in maximum width, and was still sexually immature, although the uterus coils were partially developed. The other trematodes present were also sexually immature.

The tissue defence of this host was so weak that none of the parasites had become encapsulated; this resulted in the extensive tissue destruction and subsequent death, due to the unchecked migration of the parasite.

Sheep No. 4 died on the 150th day after a period of poor general condition but without marked cachexia. Post-mortem examination revealed the cause of death to be a diaphragmatic rupture. One lobe of the liver was parasitized by three trematodes, 3.8 cm. by 2.1 cm., 3.7 cm. by 1.7 cm., and 3.0 cm. by 2.2 cm. in size. The first two were almost sexually mature but were not producing ova. They were not encapsulated and were causing severe damage to the tissue of the lobe. The third specimen was partially encapsulated by fibrous tissue, and had reached sexual maturity, sixteen eggs being found in the gall bladder. Examination of the intestinal contents revealed the presence of five normal eggs. That this specimen had reached the stage of egg production very recently is evidenced by the facts that very few eggs were present in the bile and intestinal content, the uterine coils were not fully formed, and previous anti-mortem fecal examinations had not revealed ova. Thus *F. magna* may develop to sexual maturity in *Ovis aries* in a period of somewhat less than 150 days.

Sheep No. 3 is still living on the 166th day after ingestion of the metacercariae, but is cachectic and very weak. No ova can yet be found by fecal examination. Clinical studies are being made upon this animal.

The tissues from Sheep 2 and 4 are in course of preparation for histopathological study; however, from present macroscopic observations certain features are significant. *F. magna* is a very pathogenic parasite of this host owing to the fact that the tissues are unable successfully to combat its progress in the liver. This weak tissue reaction also enables the parasite to reach sexual maturity in lightly infested hosts and the ova are able to reach the alimentary canal by way of the bile ducts. *Ovis aries* must at present be regarded as a definitive host which enables the trematode to continue its life cycle, and thus it is able to play a role similar to that of the Cervidae.

PLATE I

11

FIG. 1. *Fascioloides magna* (*Bassi, 1875*). *A mature specimen from an open cavity in the liver of Cervus canadensis. Actual length = 6.2 cm.* FIG. 2. *Fasciola hepatica* (*Linn, 1758*) *from the liver of a sheep in Canada.* (*Figs. 1 and 2 are on an equal scale to show the comparative size of these trematodes*). FIG. 3. *Young marita of F. magna from the liver of a rabbit 30 days after infestation* (×*28.5*). FIG. 4. *A marita of F. magna found in the peritoneal cavity of a rabbit 28 days after ingestion of encysted metacercariae* (×*28.5*). FIGS. 5, 6 and 7. *Fossaria parva* (*Lea*). FIG. 8. *F. parva* (*natural size*). FIG. 9. *Stagnicola palustris nuttalliana* (*Lea*). FIG. 10. *S. palustris nuttalliana* (*natural size*). FIG. 11. *A permanent habitat of Stagnicola palustris nuttalliana. The arrow points to a typical habitat of Fossaria parva in the background.* (*Photograph by Carsell, Wainwright, Alberta.*)

PLATE II

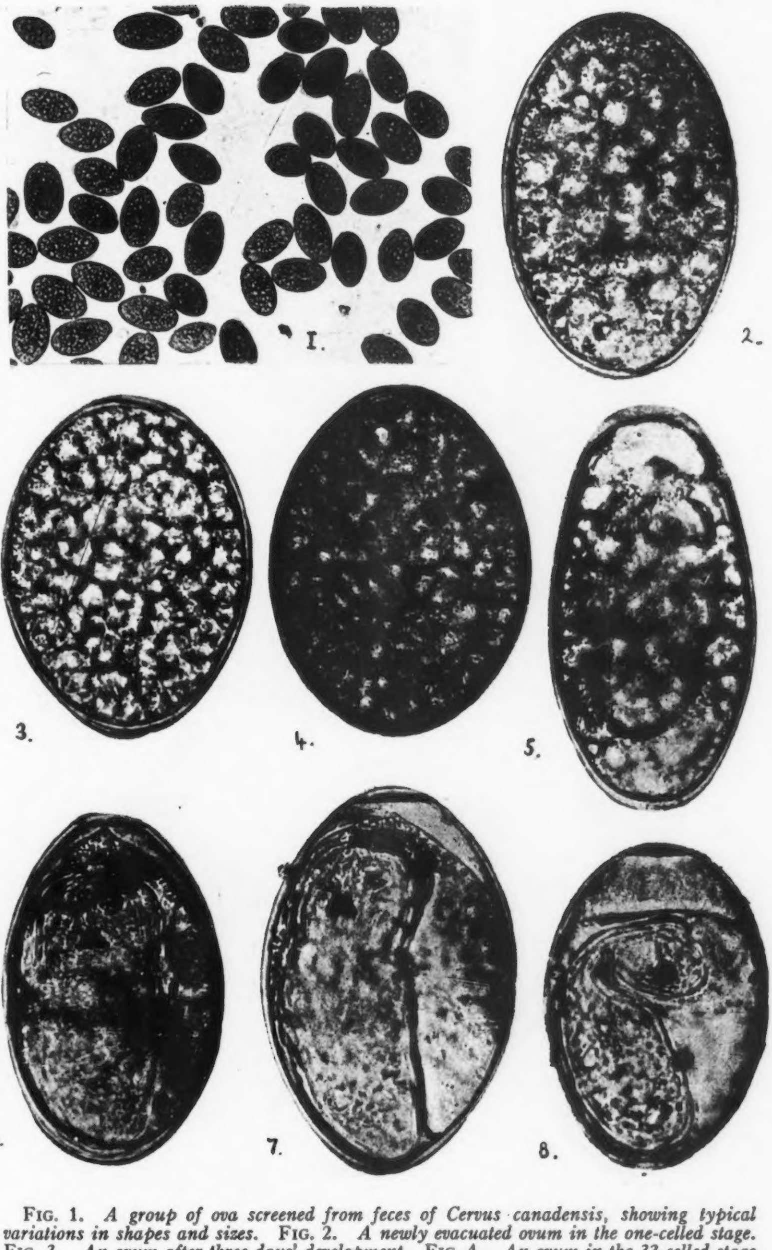

FIG. 1. *A group of ova screened from feces of Cervus canadensis, showing typical variations in shapes and sizes.* FIG. 2. *A newly evacuated ovum in the one-celled stage.* FIG. 3. *An ovum after three days' development.* FIG. 4. *An ovum in the 32-celled stage on the eighth day of development.* FIG. 5. *The morula stage.* FIGS. 5-8. *The development of the miracidium; Fig. 8 shows the typical position of a mature miracidium within the ovum.*

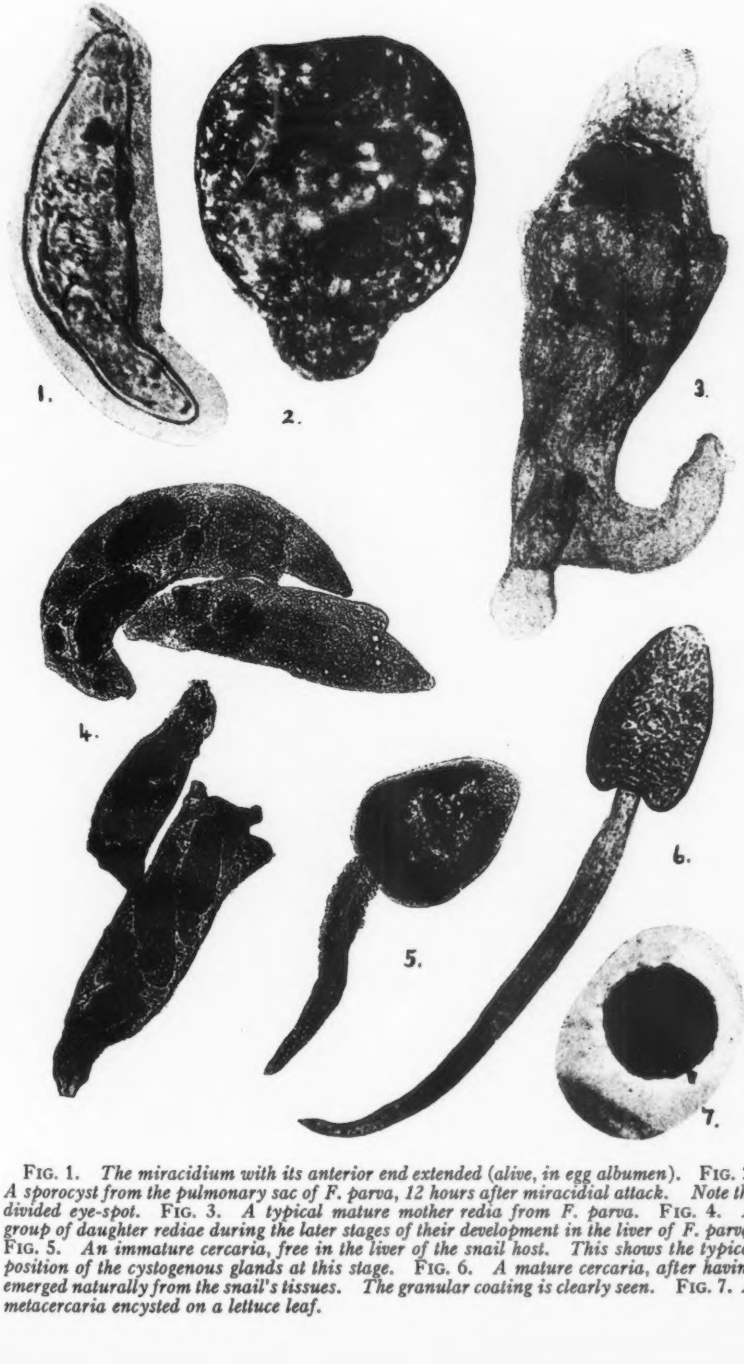

FIG. 1. *The miracidium with its anterior end extended (alive, in egg albumen).* FIG. 2. *A sporocyst from the pulmonary sac of F. parva, 12 hours after miracidial attack. Note the divided eye-spot.* FIG. 3. *A typical mature mother redia from F. parva.* FIG. 4. *A group of daughter rediae during the later stages of their development in the liver of F. parva.* FIG. 5. *An immature cercaria, free in the liver of the snail host. This shows the typical position of the cystogenous glands at this stage.* FIG. 6. *A mature cercaria, after having emerged naturally from the snail's tissues. The granular coating is clearly seen.* FIG. 7. *A metacercaria encysted on a lettuce leaf.*

PLATE IV

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FIG. 1. *An infested liver of Cervus canadensis.* FIGS. 2-3. *Sections of the same liver showing several normal cavities and a closed cyst which contained one live trematode.* FIG. 4. *A typical cavity in Odocoileus virginianus; this contained three large trematodes. Note the absence of pigmentation.*

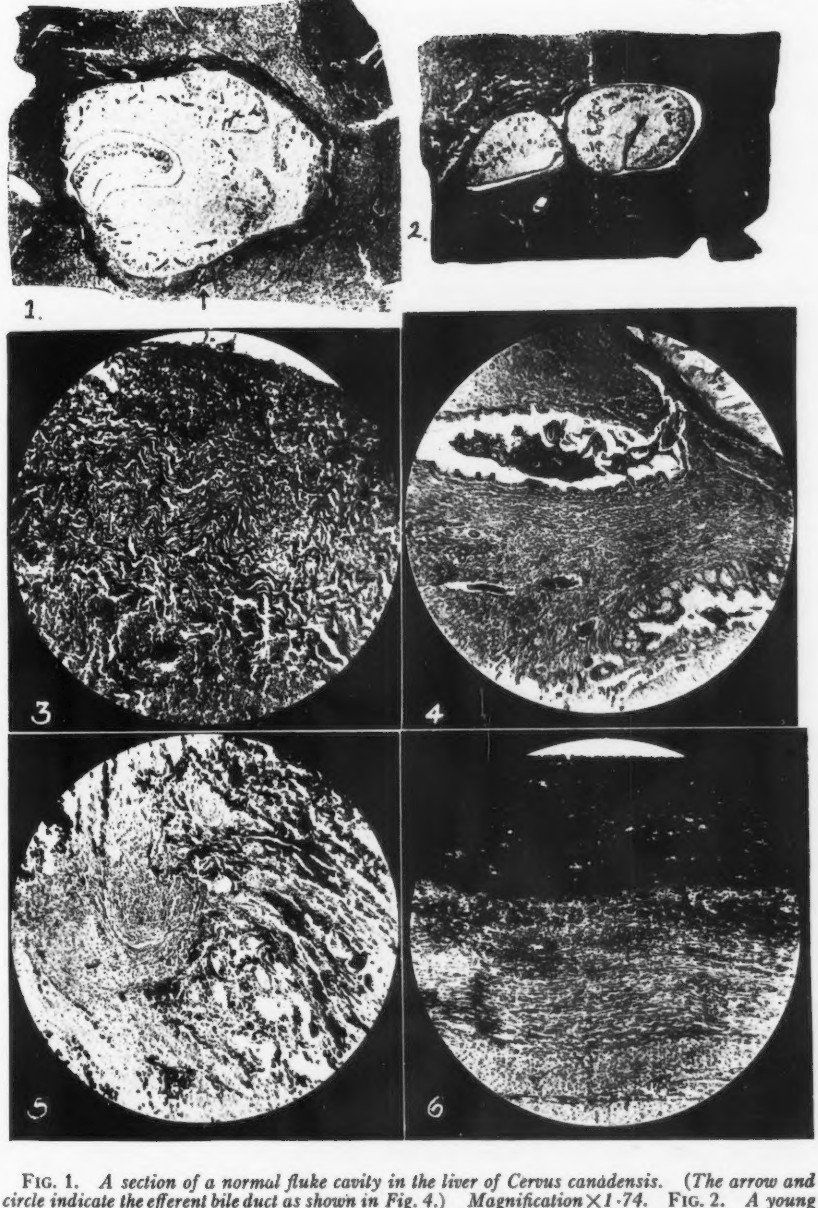

FIG. 1. *A section of a normal fluke cavity in the liver of Cervus canadensis. (The arrow and circle indicate the efferent bile duct as shown in Fig. 4.) Magnification* ×*1·74.* FIG. 2. *A young trematode in the liver of C. canadensis. The fibrous wall is being formed around the parasite. Magnification* ×*2·5.* FIG. 3. *A section through the wall of a normal cavity in the liver of O. virginianus. Note the normal liver tissue at the bottom and the successive layers of fibrous tissue. Magnification* ×*54·0.* FIG. 4. *The egress of vomitus and ova from the cavity through the open bile duct. (As Fig. 1.) Magnification* ×*30·5.* FIG. 5. *A section of the cyst wall in the liver of Bison bison, showing the inclusion of the blood-vascular system and the occlusion of the bile ducts. Magnification* ×*43·1.* FIG 6. *A section through the wall of an old closed cyst in C. canadensis. Note the character of the fibrous tissue and the adhering pigment. Magnification* ×*55·5.*

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ACTIA DIFFIDENS CURRAN, A PARASITE OF *PERONEA VARIANA* (FERNALD) IN CAPE BRETON, NOVA SCOTIA[1]

By M. L. Prebble[2]

Abstract

The immature stages of *Actia diffidens*, a tachinid parasite of the black-headed budworm, are described in detail, together with observations on the secondary integumental funnel, an ingrowth of the host body wall, within which the parasite maggots live in the later stages. In all cases observed, the integumental funnel was attached to a restricted area on either side of the mesothorax of the host larva. As the funnel is secondarily developed, its location can hardly be determined by the oviposition habits of the parent fly. Unfortunately, very little is known of the adult stage, and nothing of its mode of oviposition.

In an investigation of the natural control of the black-headed budworm, *Peronea variana* (Fernald), a tachinid parasite of interest was encountered. The presence of the older parasite maggots within the *Peronea* larvae was evidenced by a black circular disc on the mesothorax. The disc was the external indication of a secondary integumental funnel, formed from the body wall of the host larva and enclosing the parasite maggot, the posterior spiracles of which were applied against the central aperture of the disc. The parasite was not uncommon in 1930, and of considerable importance in 1931, but difficulty was encountered in rearing the adults. Adult flies were finally reared from overwintering puparia in the spring of 1932, after the *Peronea* outbreak had subsided. They were in 1933 identified by Dr. C. H. Curran as *Actia diffidens* Curran, a species established on material from Nova Scotia and New Brunswick (2). The allotype female was collected at St. Peters, Nova Scotia, in 1930, in the area infested by *Peronea variana*.

This paper embodies results from field studies in Cape Breton, N.S., supplemented by data from dissection, and microtome sections of preserved material. Although the data are incomplete, especially with respect to adult habits and the manner of oviposition, it seemed desirable to present them at this time, inasmuch as there is no likelihood that further material will be available in the near future.

Hosts of *Actia diffidens*, other than *Peronea variana*, are not known at present.

Description

Adult and Egg

Both sexes are described by Curran (2). The egg has not been observed, nor does the writer know where it is deposited. Many hundreds of *Peronea* larvae were examined during the two seasons, but only a very few dipterous eggs were noted (these were on full grown larvae). These eggs produced maggots which lived in the host larvae and pupae, without the formation of

[1] *This paper is on part of a project carried out by the Dominion Entomological Branch, the study of an outbreak of Peronea variana Fernald in the balsam and spruce forests of Nova Scotia, an account of which is now being prepared for publication.*

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an integumental funnel, so it is quite certain that they were of another species. Nor were egg shells found on *Peronea* larvae known to be parasitized by *Actia diffidens*. It seems quite improbable, therefore, that the eggs are laid externally on the host larvae, as are those of some other species of *Actia*.

First Instar (Figs. 4, 5 and 30)

The first-instar maggot ranges from .5 mm. to .7 mm. in length, is white, and tapers towards the extremities. Two pairs of sensory organs occur on the head, and are similar to those of the third instar; the anterior sensory organ has a diameter of about .006 mm.; the posterior sensory organ consists of two larger papillae and four or five smaller papillae. Anterior spiracles absent; posterior spiracles not evident on cleared skins under oil immersion lens, though a pair of minute tubes, presumably the dorsal tracheal trunks, were observed in the caudal segment; stigmatic chambers not observed on extremities of tubes, so it is doubtful if posterior spiracles are functional in the first instar.

The buccopharyngeal armature (Figs. 4 and 5) has a length of .07 mm. to .09 mm. The median tooth is broadly rounded on the dorsal side, nearly straight on the anterior margin, and produced into a short acute tip ventrally. Posteriorly the armature branches into two symmetrical arms, each with a dorsal and ventral wing; these wings are much smaller than those of other primary tachinid larvae. The cast-out buccopharyngeal armature is similar to that of young maggots, so there is no progressive sclerotization, such as is characteristic of some other tachinid species. The anterior third is less heavily sclerotized than the remainder of the armature. A small lance-like sclerite, the "lateral anterior plate" of Thompson (8) occurs on each side of the median tooth.

Spinules occur in 11 transverse bands, as follows: The first band is near the junction of the head and prothorax, and encircles the body; it consists of six or seven interrupted rows of very minute spinules, barely visible under the high power of the microscope; the spinules in the first few rows on the venter are larger than the others. The second transverse band consists of eight or nine interrupted rows of spinules, and encircles the body from the posterior region of the prothorax to about the middle of the mesothorax; the spinules in the anterior two or three rows are the heaviest, and are directed to the rear. The third band consists of about five rows of spinules, and encircles the body at the junction between the mesothorax and metathorax. The fourth band occurs at the junction of the metathorax and first abdominal segment; on the ventral side there are five or six rows of spinules (those of the first two rows being larger), but only one or two interrupted rows of spinules extend to the dorsum.

The next six bands (fifth to tenth, inclusive) occur at the margins between successive abdominal segments from the first to the seventh; they are practically confined to the venter, only a few small spinules occurring on the sides. Each band is broken into an anterior and posterior series, with a smooth space

between (see Fig. 30, A). The anterior series of each band consists of three or four rows of spinules, of which those in the first one or two rows point forward, and those in the following rows point to the rear. Usually the spinules of two adjacent rows in the anterior series are heavier than the rest, those in the first heavy row pointing forward, those in the next row pointing backwards. The posterior series of each band consists of three or four broken rows of small spinules, all of which point to the rear.

The eleventh band occurs at the junction between the seventh and eighth abdominal segments (Fig. 30, B). The band encircles the body, and consists, on the ventral side, of four or five rows of spinules (those of the anterior row are small, those of the following rows are much larger), but on the dorsal side there are only one or two rows of spinules. About midway along the lower side of the eighth abdominal segment there occur two or three irregular rows of spinules. Practically all spinules on the final segment point forward, the largest, those on the venter, being about .004 mm. long.

Second Instar (Figs. 6, 7, 19 and 31)

The second-instar maggot ranges from less than 1 mm. to about 3 mm. in length. The head is small, almost hemispherical, and the sensorial areas are similar to those of the third instar. Anterior spiracles were not observed, even under the high power of the compound microscope; their rudiments may be present in the hypoderm, but in all probability they are not functional till the second moult. The posterior spiracles are borne on the caudo-dorsal region of the final segment. Each spiracle (Fig. 19) consists of two minute circular openings, and has a maximum width of about .025 mm. The stigmatic chambers are short, of about the same diameter as the dorsal tracheal trunk, yellow, and granular in appearance. The posterior spiracles are typically separated by four or five times their width, though in some specimens they are much farther apart, and in others much closer together. No anal opening was observed. Presumably the hind gut has no connection with the exterior until the second moult.

The buccopharyngeal armature (Figs. 6 and 7) has a length of .12 mm. to .13 mm. The paired oral hooks have a long acute anterior tooth, a stouter posterior ventral tooth, and a caudo-dorsal extension above the point of articulation with the pharyngeal sclerite. The pharyngeal sclerite has an anterior bar-like extension which articulates with the oral hook of the same side, while posteriorly it bifurcates into a stout dorsal wing, and a ventral wing which is sclerotized only on the dorsal margin. The dorsal wing has a short anterior projection. The ventral trough of the pharynx is lightly sclerotized. The plate of the salivary duct lies somewhat below the level of the pharyngeal sclerites (Fig. 7).

Eleven transverse bands of spinules occur on the ventral region, near the margins between successive segments. Each transverse band, with the exception of the first, occupies the posterior region of the preceding segment, and the anterior region of the following segment. The first band occurs

between the larval head and the prothorax, the eleventh along the margin between the seventh and eighth abdominal segments. The first band consists of a single row of indistinct, backwardly directed spinules, and extends part way up the side of the larvàl head. The second, third and fourth bands consist of four interrupted rows of spinules, directed caudad; frequently these rows are reduced to three on the sides, and the spinules are noticeably smaller, extending about half-way up the sides. The fifth band (at the margin between the first and second abdominal segments) consists of three rows of spinules; on the venter there is a smooth region between the second and third row of spinules. All the spinules point to the rear. The sixth band is composed of a short anterior ventral row of very minute spinules, pointing forwards, followed by four rows of larger spinules which point backwards; there is a smooth region between the third and fourth rows; on the side there are two anterior rows of minute forwardly directed spinules. The seventh band consists of a short anterior row of minute, forwardly directed spinules, followed by three rows of larger spinules which point to the rear; there is a clear space between the third and fourth rows, and on the sides another short anterior band of forwardly directed spinules is present. The eighth band (Fig. 31, A) consists of three anterior rows of very minute, forwardly directed spinules, followed by three or four rows of larger spinules directed to the rear; the rows are broken toward the upper extremities, and the spinules are smaller. The ninth band is essentially similar to the eighth; and the tenth band is similar, except that four anterior rows of forwardly directed spinules are present instead of three. The eleventh band (Fig. 31, B) completely encircles the body between the seventh and eighth abdominal segments, and the spinules all point forward; in the ventral region there are two or three anterior rows of small spinules, followed by three or four rows of much larger spinules; on the sides and dorsum the spinules are smaller. A few small spinules occur about halfway between the heavy ventral spinules and the posterior extremity of the eighth segment.

The largest spinules in the anterior bands are about .003 mm. long, while the stout spinules at the base of the eighth abdominal segment are about .007 mm. long. They are shown enlarged in Fig. 31.

In some of the older second-stage maggots the first ten bands of spinules are difficult to detect, and in others they were not distinguished at all. The anterior bands of spinules were never detected on third-stage maggots, although the band around the margin between the seventh and eighth abdominal segments is well developed, and spinules are present in profusion about the posterior spiracles.

Third Instar (Figs. 8 to 18)

The third-instar maggot (Fig. 10) ranges from 3 mm. to over 5 mm. The larval head is small, almost hemispherical and bears two pairs of circular sensorial areas. The anterior pair are the larval antennae, or "anterior sense papillae" of Snodgrass (7); they are slightly elevated above the head

wall. The posterior pair are presumably the maxillary organs, or "posterior sense papillae" of Snodgrass. Seven or eight minute pits were noted in the circular papillae of some specimens, but were indistinguishable in others. The oral hooks lie in lateral pouches of the oral cavity, separated by an indistinct dorsal ridge.

The anterior spiracles are borne in depressions on the sides of the prothorax, near the posterior margin. Each spiracle (Fig. 12) is flattened in a vertical plane, and has five to eight orifices at the end of finger-like projections; the most common number of openings is six, but there often is asymmetry in specimens. The stigmatic chambers are yellow, and granular in appearance, and of smaller diameter than the dorsal tracheal trunks.

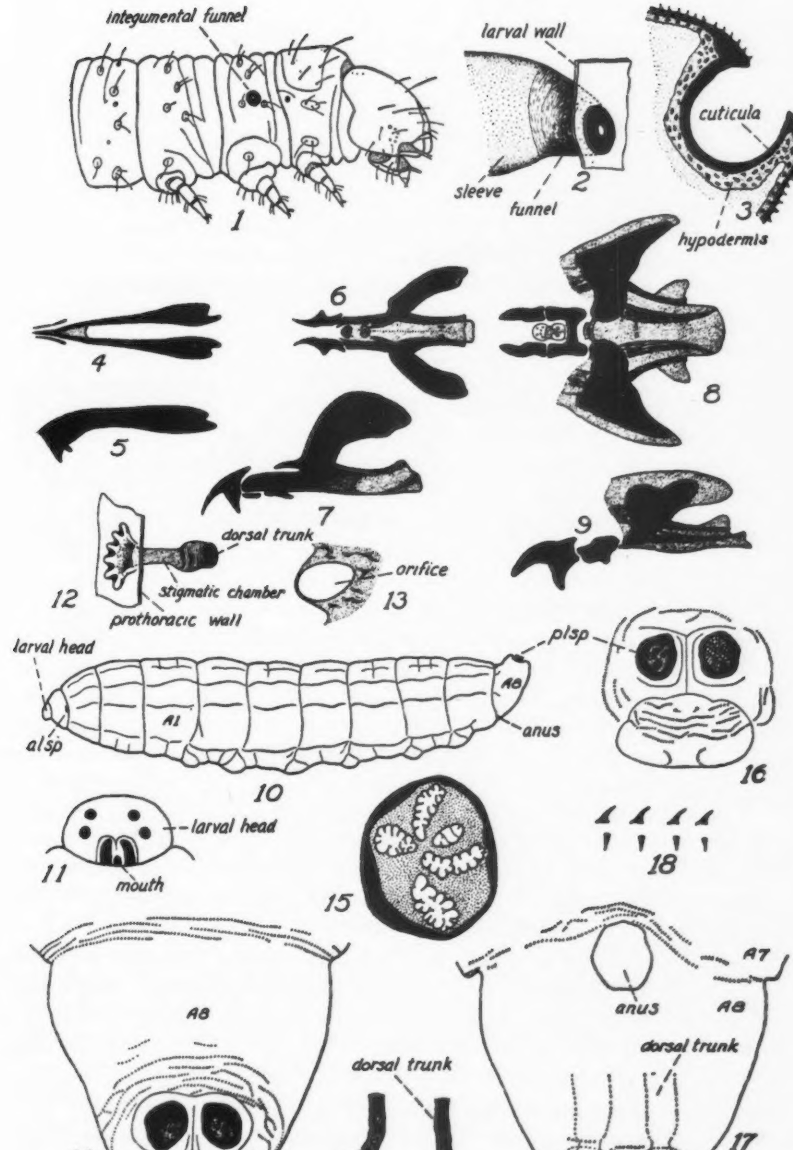
The posterior spiracles are located in a shallow depression on the tip of the eighth abdominal segment, which curves dorsally to fit snugly into the base of the integumental funnel. The stout cylindrical peritremes project above the contour of the segment, and have a diameter of .12 mm. to .17 mm.; they are separated by about half their diameter. Each spiracle has five oval to linear openings, surrounded by dark rims from which curved or forked processes are directed inwards. Usually the lateral stigmatic plate is considerably smaller than the other four.

The anus is situated on the ventral surface of the eighth abdominal segment, near the anterior margin.

The buccopharyngeal armature (Figs. 8 and 9) has a total length of about .34 mm. The oral hooks have a blunt anterior tooth and a sharper posterior ventral tooth; there is a concavity on the caudo-dorsal margin, into which the anterior ends of the lateral arms of the hypostomal sclerite fit. The hypostomal sclerite is typically H-shaped viewed from above, the transverse bridge connecting the lateral arms on the ventral side. The posterior portion of the buccopharyngeal armature consists of a lightly sclerotized ventral trough, supported on each side by the lateral pharyngeal sclerites, whose form is illustrated in Figs. 8 and 9.

Spinules occur only at the posterior end of the body, where they are arranged in two circular bands, the first near the junction between the seventh and eighth abdominal segments, and the second band about the posterior

FIG. 1. *Head and first four body segments of mature Peronea variana larva, showing location of secondary integumental funnel in side of mesothorax.* FIG. 2. *A portion of the mesothoracic wall, showing the basal portion of the funnel.* FIG. 3. *Transverse section through the anterior portion of the disc and funnel, showing continuity of cutaneous layers.* FIG. 4. *Dorsal view of the buccopharyngeal armature of the first-stage Actia maggot.* FIG. 5. *Lateral view of the same.* FIG. 6. *Dorsal view of the buccopharyngeal armature of the second-stage Actia maggot.* FIG. 7. *Lateral view of the same.* FIG. 8. *Dorsal view of the buccopharyngeal armature of the third-stage Actia maggot (lateral wings spread out somewhat from normal position).* FIG. 9. *Lateral view of the same.* FIG. 10. *Lateral view of the third-stage Actia maggot; alsp, anterior larval spiracle; plsp, posterior larval spiracle.* FIG. 11. *Ventral view of larval head, third-stage maggot (enlarged).* FIG. 12. *A portion from the prothoracic wall of the third-stage maggot, showing anterior spiracle and the stigmatic chamber (enlarged).* FIG. 13. *An orifice of the anterior spiracle of the third-stage maggot (very greatly magnified).* FIG. 14. *Dorsal view of tip of abdomen, third-stage maggot (enlarged).* FIG. 15. *View of right posterior spiracle (greatly enlarged).* FIG. 16. *Enlarged caudal view of tip of abdomen, showing spiracles, spinules, and sensory setae.* FIG. 17. *Ventral view of tip of abdomen, third-stage maggot.* FIG. 18. *Spinules of the third-stage maggot viewed from side (upper) and from above (lower).* FIG. 19. *Posterior spiracles and stigmatic chambers of second-stage maggot.*


integumental funnel
1
larval wall
sleeve
funnel
2
cuticula
3
hypodermis
4
5
6
7
8
9
dorsal trunk
stigmatic chamber
prothoracic wall
12
orifice
13
larval head
alsp
A1
A8
plsp
anus
10
16
larval head
mouth
11
15
18
A8
14
dorsal trunk
stigmatic chamber
19
A7
anus
A8
dorsal trunk
17

spiracles (Figs. 14, 16 and 17). The spinules are about .005 mm. in length, triangular when seen from above, but curved and sharp seen in side view (Fig. 18). As in preceding instars, the arrangement of the spinules varies somewhat, but the anterior band consists of three or four interrupted rows of spinules, which are fairly regular on the dorsum (Fig. 14), and more irregular on the venter (Fig. 17). Some of the spinules in front of the anus may be directed posteriorly, but nearly all other spinules in the anterior band point cephalad. The posterior band consists of about four irregularly broken rows of spinules encircling the truncate tip of the abdomen (Fig. 14); the spinules are more numerous above and below the spiracles, and rather sparse laterally. Nearly all the spinules in the posterior band point cephalad, that is, with the apices directed away from the posterior spiracles. The spinules below the spiracles are borne on an oval elevation (Fig. 16). On the same elevation there occur two minute sensory setae; these were not observed on the preceding instars.

Four longitudinal rows of creases extend from the second thoracic to the seventh abdominal segment (Fig. 10); these creases are also noticeable in the puparium. Six transverse ambulatory elevations of the body wall occur on the venter, between successive abdominal segments, the first occurring between the first and second abdominal segments, the last between the sixth and seventh segments. Less prominent elevations occur on the anterior segments.

The Puparium (Figs. 24 to 27)

The puparium ranges from 2.8 mm. to 4.5 mm. in length, and from 1.3 mm. to 2.0 mm. in width. Yellow at first, it soon turns brown. Eleven segments are distinguishable, the first being the prothorax; apparently, as in *Rhagoletis pomonella* Walsh (7), the larval head is involuted through the anterior opening. The anterior end is rounded, and is marked by horizontal and transverse creases which represent the lines of cleavage of the puparial cap. The horizontal line arches over the mouth opening (Fig. 25), thence back below the prothoracic spiracles to the anterior margin of the first abdominal segment, where it meets the transverse line which encircles the puparium. The transverse line is heavier on the dorsal half, and the dorsal half of the puparial cap is usually pushed off at emergence.

The anterior spiracles (Fig. 26) are flattened, inconspicuous, and consist usually of six lobes, though the number is variable as in the larva. The dorsal half of the prothorax, above the horizontal cleavage line, is marked by about seven faint creases which give it a rugose appearance. The mouth opening is marked by an oval scar at the centre of the prothorax (Fig. 25).

The posterior spiracles project slightly above the general contour (Figs. 24 and 27). The anus is visible as a circular scar at the anterior margin of the final segment, and from a narrow slit in the scar there projects inward a short stalk, the remnant of the larval proctodeum. There are two protuberant areas on the venter of the final segment, one bearing the anus, the other representing the elevated and spinulated region described for the third instar.

Abdominal spiracular openings could not be detected on overwintering puparia, or on puparia from which the flies had emerged. There is, however, on each side of the first abdominal segment, near the posterior margin, a circular opening (Fig. 24) through which the prothoracic cornicles of the pupa (Fig. 23) are thrust.

The longitudinal creases of the third-instar maggot appear on the puparium, as do also the remnants of the ambulatory elevations. The latter have the form of narrow rugose areas at the margins between successive abdominal segments, between the first and second segments, to the sixth and seventh segments.

The surface of the puparium is faintly rugose transversely, and this is more pronounced on the final segment. The larval spinules of the final segment are visible under high magnifications.

Prepupa

Within puparia which were opened while the insect was still in the pupal stage, was found a fine transparent membrane, lying snugly against, but separable from, the puparial wall. The cast-out buccopharyngeal armature of the third instar, the linings of the dorsal tracheal trunks, and the proctodeal lining, lie on the inner surface of this membrane, that is on the surface exposed when viewed in a cut-open puparium. This membrane is similar to the prepupal cuticula of *Rhagoletis pomonella*, concerning which Snodgrass writes as follows: "Now, from reading most descriptions of fly metamorphoses, we should expect to find a pupa within the puparium. But the object disclosed in the puparial capsule of the apple maggot at this stage has no character suggesting a pupa . . . . The new creature, moreover, has a cuticle distinctly its own . . . . In fact, it is evident that the maggot of the third instar has changed by a puparial molt, not to a pupa, but to a *fourth* instar larva, an intra-puparial, prepupal larval stage, complete in all respects except for the retention of the stomodeal, proctodeal and tracheal linings of the third larval instar . . . . The larva of *Rhagoletis* remains in this post-puparial instar a varying length of time, but usually not beyond the end of 48 hours from the time it entered the ground. Then begins another molt which ends in the complete shedding of the papillated cuticle and in the disclosure of the true pupa."

Though no puparia of *Actia diffidens* were opened before the pupa was formed, that is, while the insect was in a prepupal stage, it appears quite conclusive, from the presence of this membrane and its relation to the cast-off chitinous parts within it, that there is a prepupal stage, corresponding in its essential features, to that which occurs in *Rhagoletis*.

Pupa

A small number of puparia were cut open in October, about two months after being formed, and the pupae were in the stage of development illustrated in Figs. 20 to 22. The insect probably overwinters in this, the phanerocephalic pupal stage. The eyes were not distinguished, nor were the abdominal segmentation, or the abdominal spiracles, evident on uncleared specimens.

The anterior pupal spiracles (Fig. 23) consist of a pillar-like prothoracic cornicle, borne on a high mound of the body wall, and a two-lobed inner spiracle, borne on a smaller elevation. The prothoracic cornicle has a simple orifice, which is thrust into the air through the opening on the first abdominal segment of the puparium. The inner spiracle has two lobes, each of which carries over twenty small oval orifices; it communicates with the interior of the puparial chamber. The yellow, granular, stigmatic chamber of the pupal spiracle is separated into two parts, a large ovoid chamber beneath the prothoracic cornicle, and a two-lobed chamber beneath the inner spiracle, which communicates posteriorly with the dorsal tracheal trunk.

Life History

Adults emerged the last few days of May and the first of June at the Fredericton laboratory. Probably emergence is later in the field. Adults were taken in flight in Nova Scotia during the middle part of June.

Parasite maggots were recovered in small numbers from *Peronea* larvae which had been isolated in the third stage, and in larger numbers from *Peronea* larvae isolated in the fourth and fifth stages. Judging by the occurrence of the host larval stages in Cape Breton, parasitism commences late in June, and is heaviest the first half of July. The full-grown maggots issue from the dead hosts from the middle of July to the middle of August, drop to the forest floor and form their puparia.

The results obtained from dissections of host larvae are as follows: *(a) Peronea* larvae in which no funnel was developed: First- and second-stage *Actia* maggots were found in fourth- and fifth-stage host larvae, living *freely* in the body cavity from the prothorax to the tenth abdominal segment; *(b) Peronea* larvae in which the funnel was developed: Second-stage *Actia* maggots were commonly found in funnels, and all third-stage maggots were dissected from funnels. When the enclosed maggot was in the third stage, the buccopharyngeal armature of the second stage was always lodged in the base of the funnel. In a very few instances the buccopharyngeal armature of the first stage was also found in the base of the funnel, showing that the funnel had developed before the first moult; but usually the armature of

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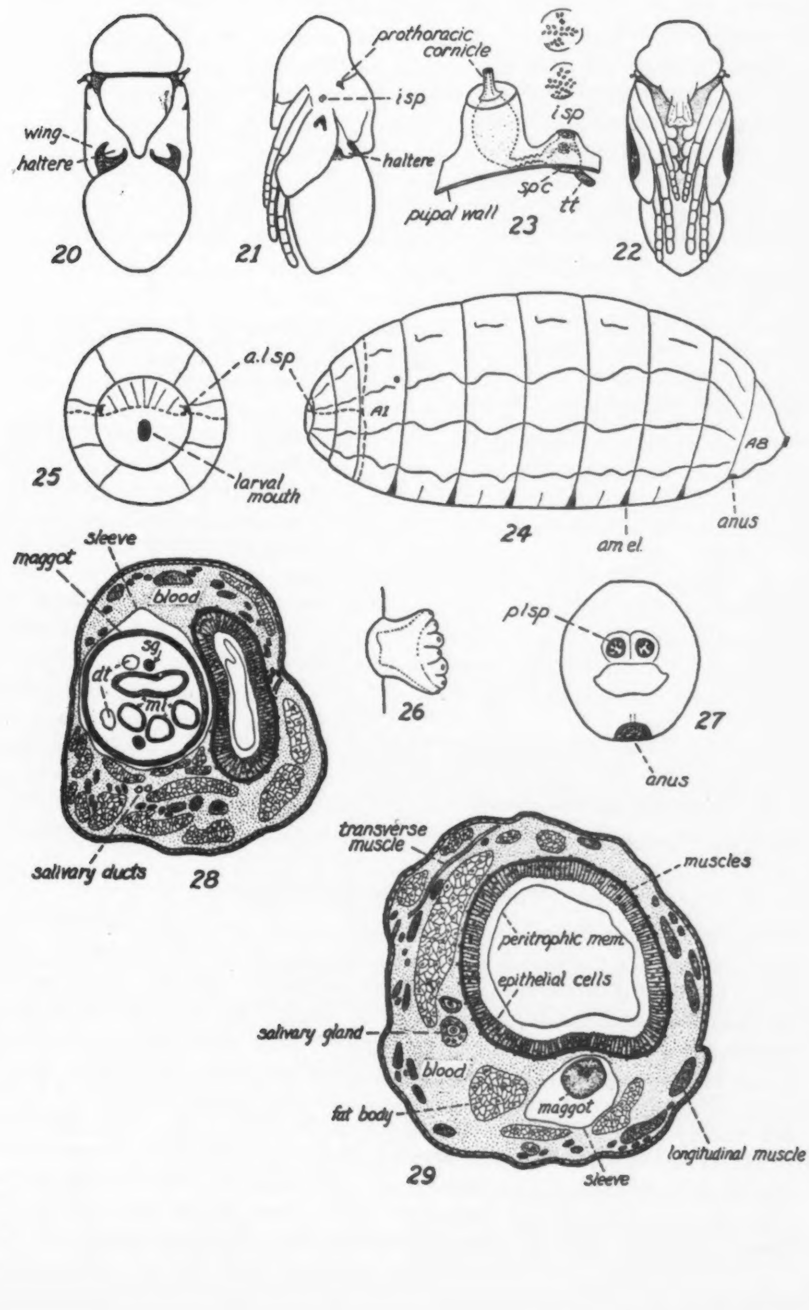
FIG. 20. *Dorsal view of overwintering pupa.* FIG. 21. *Lateral view of the same; isp, inner spiracle.* FIG. 22. *Ventral view of the same.* FIG. 23. *A portion of the pupal wall, showing prothoracic cornicle and inner spiracle, isp (enlarged above), stigmatic chamber, spc, and dorsal tracheal trunk, t.t. (enlarged).* FIG. 24. *Lateral view of puparium (enlarged); alsp, anterior larval spiracle, am, el, remains of larval ambulatory elevations.* FIG. 25. *Enlarged anterior view of prothorax and mesothorax of puparium.* FIG. 26. *Anterior view of anterior larval spiracle on puparium (greatly enlarged).* FIG. 27. *Enlarged posterior view of final segment of puparium, showing posterior larval spiracles, plsp, and anus.* FIG. 28. *Transverse section through the fore part of the abdomen of a parasitized fifth-stage Peronea larva, showing the Actia maggot within the sleeve. The microtrichia of the host cuticula have been omitted (see* FIG. 3*), as well as many of the Actia organs; dt, dorsal tracheal trunks of the maggot; sg, salivary gland of the maggot; mi, four cuts through the convoluted mid-intestine of the maggot.* FIG. 29. *Transverse section farther back than that shown in Fig. 28; cut through the head of the maggot (note the oral hooks). The pharynx and other organs of the maggot have been omitted in the drawing.*


wing
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23
22
a.l.sp
larval mouth
25
A1
A8
anus
am el.
24
sleeve
maggot
blood
sg
dt
ml
salivary ducts
28
26
p.l.sp
27
anus
transverse muscle
muscles
peritrophic mem.
epithelial cells
salivary gland
blood
fat body
maggot
longitudinal muscle
sleeve
29

the first stage was outside the funnel, lodged against the body wall or alimentary tract of the host larva, from the prothorax as far back as the ninth abdominal segment.

From the above it is apparent that the integumental funnel is developed secondarily, not at the point of entrance of the newly hatched burrowing maggot, but rather as a response to irritation from within, which most often occurs during the second stage of the parasite, and the fifth stage of the host. The large hooked spinules on the posterior segment of the first- and second-stage *Actia* maggots are probably employed in rupturing the body wall, and in anchoring the maggot in the developing funnel. In one instance the funnel appeared in a host larva isolated for some days, and eight days later the full-grown maggot issued from the dead host.

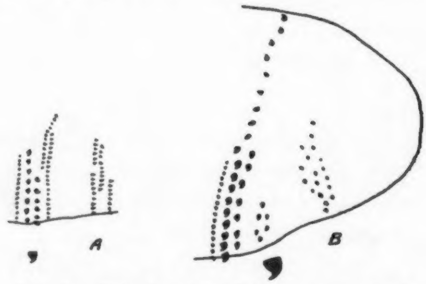

FIG. 30. *Spinules of the first-stage maggot. A. Those of the eighth transverse band, between the fourth and fifth abdominal segments. B. Those of the final abdominal segment.*

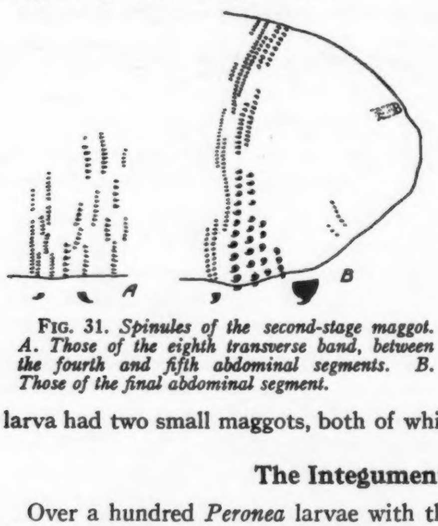

FIG. 31. *Spinules of the second-stage maggot. A. Those of the eighth transverse band, between the fourth and fifth abdominal segments. B. Those of the final abdominal segment.*

Of approximately one hundred *Peronea* larvae parasitized by *Actia*, which were dissected, only one contained more than one parasite maggot. This host larva had two small maggots, both of which were free in the body cavity.

The Integumental Funnel

Over a hundred *Peronea* larvae with the funnel have been examined, and in every instance the funnel was located on one side of the mesothorax (Fig. 1). The point of attachment was always within a restricted area, between the seta *theta* and the *kappa* group, either slightly above or below the horizontal line between these setae, or in some cases right on the pinaculum which bore the setae. The black disc is a thickened area in the host integument (Figs. 2 and 3), consisting of cuticula and hypodermis; these layers are readily distinguished in the basal part of the funnel. The ingrowing funnel bends abruptly and continues backward as a fine sleeve to one side of the alimentary tract. The hypodermal cells were not distinguished in the thin sleeve, under the oil immersion lens. The maggot rests within the sleeve, with the posterior segment curved into the basal funnel and the

posterior spiracles against the aperture. Whether the sleeve is closed at the free end is uncertain, but at any rate it has been observed in microtome sections beyond the tip of the maggot.

The early stages of *Actia* do little damage to the host, and even in the first part of the third instar, the chief abnormality seems to be crowding of the host intestine by the growing parasite (Fig. 28). During the last two or three days within the host, the maggot feeds at a destructive rate, and consumes nearly all the organs and tissues, except the tracheae and occasionally the alimentary tract. A day or more after the host has been killed, the maggot issues from the remains (through the anus in some cases that were observed), leaving the integumental funnel attached to the mesothorax.

A perplexing feature of the host-parasite relationship is the apparently invariable selection of the side of the mesothorax for the location of the secondary funnel. It may be that the young free-living maggot, when moved to establish a respiratory connection with the exterior, is governed by the absence of spiracles and the tracheal trunks leading from them, which would limit selection to the mesothorax, metathorax, and the last two abdominal segments. Of these four, the mesothorax would be most suitable as a point of attachment, as ample room for growth would be assured to the parasite, which would lie with its mouth near the source of rich nutriment, that is, near the region of the mid-intestine of the host. This, however, is only speculation.

Acknowledgments

The writer is pleased to express his indebtedness to Dr. W. H. Brittain of Macdonald College, Quebec, and Mr. R. E. Balch of the Dominion Entomological Branch, who in numerous ways facilitated the progress of this work.

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INFLUENCE OF WEATHER CONDITIONS ON THE NITROGEN CONTENT OF WHEAT[1]

By J. W. Hopkins[2]

Abstract

A statistical study of results from experimental plots of Marquis wheat grown annually (though not on the same soil) at five points in Saskatchewan and Alberta indicates that there was a significant negative correlation between the amount of rainfall during the growing season and the nitrogen content of wheat. The main effect of rainfall was exerted during May and June. The data do not justify the conclusion that the amount of rain falling in July or August, or the amount of pre-seasonal precipitation, modified the nitrogen content significantly. Mean maximum temperature for July or August failed to show a significant correlation with nitrogen content, but may not be a satisfactory measure of the temperature conditions actually experienced by the crop.

It is suggested that the preponderating effect of early rainfall may be due to the fact that it stimulates tillering and vegetative development generally. The available nitrogen must thus be distributed amongst an increased number of culms, whilst at the same time the total leaf area devoted to the production of carbohydrates is augmented.

1. Introductory

The present surplus of agricultural commodities has caused increased attention to be directed to the quality of Canadian wheat as a factor affecting export sales, with the result that information respecting the quality of the annual production may be as important as the statistics of quantity.

A high protein content is the most important single concomitant of wheat strength. It is generally conceded that weather conditions exert an important influence on the composition of the grain, as well as on the yield but, as yet, the quantitative aspects of these relations have not been definitely established for Canadian crops.

2. Experimental Data

Observational results were acquired through the courtesy of Dr. F. T. Shutt, who inaugurated, in 1912, an extensive experiment on the influence of environment on wheat quality. Each year, parent seed of the variety Marquis O-15, produced during the previous season at Indian Head, Saskatchewan, was distributed to Dominion Experimental stations throughout the country, sown in small plots, and five-pound samples of the resulting grain harvests sent to Ottawa for analysis. An account of the results obtained during the period 1912–1932, when the experiment was terminated, has been given by Shutt and Hamilton (6), together with certain conclusions deduced from variations in the average protein content of the grain produced at different stations. The results at individual stations in different seasons may, however, be utilized to study more directly the influence of weather conditions.

Manuscript received November 22, 1934.

[1] *Contribution from the Division of Biology and Agriculture, National Research Laboratories, Ottawa. Published as Paper No. 63 of the Associate Committee on Grain Research.*

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From 1915 onwards, extensive notes on the previous history of the experimental plot, growth of the crop, incidence of frost, hail and disease, etc., as well as the total yield of grain secured, were made in a uniform manner at all stations. These proved to be of great value in the discovery of certain sources of variation and the elimination of results secured under obviously abnormal conditions.

TABLE I

YIELD OF GRAIN (LB. PER ACRE) AND NITROGEN CONTENT (%) OF WHEAT

Station	Year	Yield	N content	Station	Year	Yield	N content
Lacombe, Alberta	1915	3520	2.59	Indian Head, Saskatchewan	1926	3020	2.35
	17	3000	2.49		28	2650	2.38
	21	3260	2.87		29	1950	2.86
	22	1200	3.24		31	1180	3.13
	25	3450	2.70		32	1250	3.02
	28	2250	2.59				
	30	3720	2.99	Scott, Saskatchewan	1915	2880	2.82
	31	3720	2.47		17	1040	3.12
	32	4340	3.09		18	280	3.55
					21	1700	3.22
Lethbridge, Alberta	1915	3840	2.69		23	1800	2.84
	16	2910	2.71		24	590	3.01
	17	1500	2.95		25	1690	2.87
	21	540	3.05		26	1220	3.22
	22	1500	2.92		29	1040	3.47
	25	1540	3.47		30	2560	2.80
	26	2290	3.03		31	1630	2.98
	27	1270	2.39		32	1310	3.00
	28	2720	2.75				
	29	1500	3.64	Rosthern, Saskatchewan	1915	2770	2.91
	30	760	2.92		21	1760	3.23
	31	760	2.92		22	2600	2.96
	32	2140	2.98		24	720	2.94
					25	1880	2.66
Indian Head, Saskatchewan	1915	3880	2.80		26	820	2.93
	18	2200	2.93		27	2050	2.55
	24	1540	2.73		28	1980	2.91
	25	2820	2.58		29	900	3.24
					30	1840	2.68
					32	2080	3.13

Considering only stations in the prairie provinces, examination of the above records revealed that the great majority of the experimental crops were grown on land which had been summerfallowed or had supported a "summerfallow substitute" crop, such as potatoes, in the previous season. At the two Manitoba stations, Brandon and Morden, however, a diversity of previous treatment occurred, including as alternatives to the elsewhere usual summerfallow the growing of crops such as wheat, oats, barley, alfalfa, sweet clover, corn, melons and white beans (manured). Since to these actual or potential sources of variation in the yield and composition of the succeeding crop must be added several severe infestations of black stem rust, it was judged that attention should be confined to the results obtained at stations in central and southern Saskatchewan and Alberta. These also were freed

of a number of crops grown in succession to legumes, of a further number affected by catastrophic occurrences, such as frost, hail or heavy disease infestation, and of still others of doubtful status owing to the absence or loss of the field records. The grain yields and nitrogen contents of the remainder, which are believed to be reasonably comparable, are shown in Table I. The meteorological data, with which these are to be correlated, were obtained from the Monthly Record of the Meteorological Service of Canada, supplemented by correspondence with the Director of that Service, and are given in Tables II, IV and V.

TABLE II

SUMMER PRECIPITATION (INCHES OF RAIN) BY MONTHS

Station	Year	May	June	July	Aug.	Station	Year	May	June	July	Aug.
Lacombe,	1915	1.25	8.28	2.79	0.84	Indian Head,	1928	0.64	6.05	2.14	0.34
Alberta	17	3.26	1.49	1.13	1.88	Saskatchewan	29	2.16	1.12	0.73	0.18
	21	1.69	1.85	3.28	0.98		31	0.23	1.18	1.87	2.38
	22	1.30	1.75	1.88	2.94		32	1.87	3.44	2.22	3.27
	25	1.53	1.51	1.32	3.87						
	28	0.50	7.30	1.66	2.56	Scott,	1915	1.40	3.54	2.11	0.48
	30	1.61	2.08	3.72	2.93	Saskatchewan	17	0.41	0.88	1.03	1.42
	31	0.86	8.11	2.59	2.53		18	0.19	0.29	1.87	0.93
	32	2.08	3.97	2.30	1.84		21	1.39	1.66	1.65	0.56
							23	0.95	5.67	4.25	1.45
Lethbridge,	15	3.03	4.84	3.44	0.96		24	1.10	0.58	0.70	2.87
Alberta	16	3.77	3.54	3.33	2.97		25	1.36	3.48	2.68	1.31
	17	0.95	1.42	1.37	2.00		26	2.97	1.39	0.75	2.52
	21	0.96	1.04	3.23	0.46		29	0.63	2.64	0.67	0.45
	22	0.89	1.87	2.30	0.40		30	0.38	2.79	2.45	1.08
	25	0.43	3.40	0.82	1.85		31	0.12	2.41	3.13	0.83
	26	0.64	4.67	1.15	2.31		32	1.82	2.83	2.78	1.30
	27	7.32	1.60	1.93	1.74						
	28	0.09	6.79	3.98	1.54	Rosthern,	1915	1.16	1.00	3.12	0.28
	29	2.63	3.72	0.52	0.59	Saskatchewan	21	1.88	1.49	4.91	0.64
	30	1.54	1.42	0.57	0.57		22	3.66	1.54	0.53	3.28
	31	1.22	1.55	1.09	0.19		24	0.70	0.87	0.77	3.73
	32	2.99	2.06	0.74	3.63		25	0.96	4.01	2.31	3.60
							26	3.38	0.48	0.79	1.48
Indian Head,	15	1.37	2.32	1.92	1.75		27	2.62	4.32	5.63	1.22
Saskatchewan	18	1.72	0.82	1.96	3.02		28	0.40	2.57	4.26	1.03
	24	0.36	2.22	1.05	3.00		29	1.13	3.05	0.72	0.81
	25	0.41	3.47	0.60	0.46		30	1.09	2.47	2.44	0.42
	26	3.07	1.99	1.25	1.97		32	0.71	3.68	3.16	2.43

3. Statistical Analysis

Seasonal Variance of Yield and Nitrogen Content

In order to eliminate any bias due to differences in average soil composition or weather conditions between stations, the quantities dealt with will be the seasonal deviations from the station averages, rather than the absolute values of yield and nitrogen content listed in Table I. Assuming the results obtained at different stations in the same year to be independent, the observations, 54 in all, provide 49 degrees of freedom for the estimation of the seasonal variance of yield and nitrogen content, and the covariance of these with rainfall, etc.

The yield of grain secured exhibits a seasonal standard deviation of 847 lb. per acre, corresponding to a coefficient of variation of 41.8%, the analogous values for nitrogen content being 0.86 and 29.6% respectively. An inspection of Table I will reveal that high yield is frequently associated with low nitrogen content and *vice versa*. There are however several exceptions to this tendency. The seasonal covariation of the two quantities yields a correlation coefficient of only -0.40, which, although significant, indicates that the fluctuations in the two attributes are far from strictly proportional.

Influence of Summer Rainfall

Table II shows the amounts of precipitation recorded at the various stations during the months of May, June, July and August of the seasons for which crop records are available in Table I. It is required to estimate from the data of Tables I and II the values of the regression coefficients b in the equation

$$(N-\bar{N}_s) = b_1(r_1-\bar{r}_{1s}) + b_2(r_2-\bar{r}_{2s}) + b_3(r_3-\bar{r}_{3s}) + b_4(r_4-\bar{r}_{4s})$$

where $(N-\bar{N}_s)$ represents the amount by which the nitrogen content in any given season is in excess or defect of the appropriate station average, and $(r-\bar{r}_{1s})$, $(r_2-\bar{r}_{2s})$, etc., represent the corresponding seasonal fluctuations of May, June, etc., rainfall. The normal equations to determine the coefficients b by the method of Least Squares are:—

$$\begin{aligned}
78.5671b_1 - 27.3152b_2 - 6.1063b_3 + 9.6777b_4 &= -4.5431\\
-27.3152b_1 + 169.8572b_2 + 40.0188b_3 - 11.3695b_4 &= -8.7296\\
-6.1063b_1 + 40.0188b_2 + 73.8206b_3 - 13.6903b_4 &= -3.6183\\
9.6777b_1 - 11.3695b_2 - 13.6903b_3 + 56.7984b_4 &= +.3672
\end{aligned}$$

giving

$b_1 = -.0804\%$ nitrogen per additional inch of May rainfall

$b_2 = -.0586\%$ nitrogen per additional inch of June rainfall

$b_3 = -.0234\%$ nitrogen per additional inch of July rainfall

$b_4 = +.0028\%$ nitrogen per additional inch of August rainfall

as the average effect of one additional inch of rain during the months specified.

Replacing the quantities on the right hand side of the above equations by the covariance of yield and rainfall, 6781.94, 31178.18, 18822.93 and -3640.17 respectively, enables the regression of grain yield on rainfall by months to be computed as follows:—

$b_1 =$ 160 lb. per acre per additional inch of May rainfall

$b_2 =$ 167 lb. per acre per additional inch of June rainfall

$b_3 =$ 175 lb. per acre per additional inch of July rainfall

$b_4 = -16$ lb. per acre per additional inch of August rainfall.

The sum of the squares of the seasonal deviations of nitrogen content and yield, corresponding to 49 degrees of freedom, may now be partitioned as indicated in Table III.

TABLE III

Variance	Degrees of freedom	Nitrogen content		Yield	
		Sum of squares	Mean square	Sum of squares	Mean square
Accounted for by regression	4	.9625	.2406	9639000	2410000
Deviations from regression	45	2.6810	.05958	25478000	566200
Total	49	3.6435		35117000	

In both cases the regression function has accounted for a greater proportion of the total variance than is consistent with the hypothesis of non-association, indicating that a real correlation does exist. But it will be noted that whereas the yield of grain secured is influenced by the amount of rain falling in May, June and July, the major, and indeed the only definitely significant, effect upon nitrogen content appears to be exerted during the first two months of the growing season. The degree of association is only moderate, being measured by multiple correlation coefficients of $R=0.52$ and $R=0.51$ in the case of grain yield and nitrogen content respectively. This is not however altogether surprising in view of the fact that the crop was grown on different plots at each station each year.

As there is some degree of correlation (negative) between the precipitation recorded in May and June of the same year during the period under review, it is to be expected that a good approximation to the regression of nitrogen content on summer rainfall might be obtained by the use of the total precipitation for May and June only. The resulting regression coefficient has in fact the value -0.068% nitrogen for each additional inch of rain, and accounts for only a slightly smaller amount (0.9090) of the total sum of the squares of annual deviations in nitrogen content than the regression equation in which all four months are treated separately (0.9625).

Influence of July and August Temperature

It is suggested by Shutt and Hamilton (6) that scanty precipitation and high temperatures during the later weeks of development and ripening of the kernel, by drying out the soil, constitute the chief environmental factor conducive to high protein content.

The average daily mean temperatures recorded for the months of July and August at any particular station show but slight seasonal variation, and such variation as does occur is wholly uncorrelated with nitrogen content.

It was felt, however, that the mean daily maximum for the month might be a better measure of the temperature conditions actually experienced by the crop. The values of this quantity are listed in Table IV.

TABLE IV

MEAN MONTHLY MAXIMUM TEMPERATURE (DEGREES F.)

Station	Year	July	August	Station	Year	July	August
Lacombe,	1915	68	79	Indian Head,	1928	76	74
Alberta	17	77	72	Saskatchewan	29	83	83
	21	76	76		31	81	78
	22	77	78		32	78	77
	25	80	72				
	28	75	70	Scott,	1915	72	82
	30	78	76	Saskatchewan	17	82	76
	31	75	73		18	80	76
	32	73	76		21	78	78
					23	77	70
Lethbridge,	1915	70	82		24	79	69
Alberta	16	75	73		25	76	74
	17	83	76		26	80	72
	21	79	78		29	79	79
	22	76	80		30	76	77
	25	79	76		31	76	74
	26	82	72		32	74	76
	27	74	73				
	28	74	72	Rosthern,	1915	70	80
	29	80	83	Saskatchewan	21	78	76
	30	80	81		22	78	77
	31	78	79		24	79	70
	32	80	77		25	78	75
					26	82	74
Indian Head,	1915	70	80		27	76	75
Saskatchewan	18	78	73		28	78	72
	24	79	72		29	80	80
	25	78	79		30	79	84
	26	83	74		32	76	76

Considering the crude data only, there is some apparent covariation of temperature and nitrogen content, above-average temperatures tending to be associated with above-average nitrogen. When, however, due allowance is made, by the method of partial correlation, for the influence of May and June rainfall, the covariance of nitrogen content and temperature is much reduced. The effect of July temperature variations is then measured by a partial regression coefficient of 0.008 ± 0.012, which is smaller than its standard error and hence statistically insignificant. The August partial regression coefficient is $0.017 \pm 0.0094\%$ nitrogen per degree F. Here there is perhaps some indication of a real effect, but as this coefficient also does not significantly exceed its standard error, no definite conclusion is justified.

Influence of Pre-seasonal Precipitation

In view of the fact that the maximum influence of summer rainfall on nitrogen content appears to be exerted in the first two months of the growing season, it might be thought that pre-seasonal precipitation would also have some indirect effect, through the initial supply of soil moisture thus made available.

The total precipitation, expressed as inches of rain, recorded from August 1 of the preceding year to April 30 of the crop year, is shown for the various stations and seasons in Table V. If a proper estimate of the regression of nitrogen content on pre-seasonal precipitation is to be obtained, allowance must be made for the effect of the associated May and June rainfall, which has already been shown to be significant. When this is done, the regression coefficient deduced is $-0.023 \pm 0.013\%$ nitrogen for each additional inch of precipitation in the period August 1 to April 30. This does not significantly exceed its standard error, so that although there is some indication of correlation, the reality of the effect cannot be said to be definitely established.

TABLE V

Pre-seasonal precipitation (inches of rain), August 1 of preceding year to April 30 of crop year

Station	Crop year	Precipitation	Station	Crop year	Precipitation
Lacombe, Alberta	1915	7.64	Indian Head, Saskatchewan	1928	12.99
	17	12.98		29	3.93
	21	6.64		31	4.53
	22	5.82		32	7.27
	25	11.72			
	28	12.22	Scott, Saskatchewan	1915	11.44
	30	7.07		17	8.48
	31	7.27		18	5.28
	32	11.79		21	10.48
				23	7.04
Lethbridge, Alberta	1915	10.51		24	5.95
	16	7.99		25	11.86
	17	14.08		26	7.61
	21	5.73		29	3.83
	22	8.12		30	5.67
	25	13.06		31	5.99
	26	10.02		32	10.15
	27	11.87			
	28	13.43	Rosthern, Saskatchewan	1915	7.08
	29	8.84		21	10.81
	30	10.11		22	7.24
	31	7.42		24	7.49
	32	9.92		25	11.41
				26	9.90
Indian Head, Saskatchewan	1915	5.97		27	8.92
	18	7.89		28	6.77
	24	7.78		29	6.05
	25	13.83		30	7.70
	26	8.85		32	9.73

It might perhaps be suggested that variations in winter snowfall have no great influence on soil moisture, and serve merely to mask any real correlation between nitrogen content and pre-seasonal rainfall. To test this point, calculations similar to the foregoing, but employing the precipitation during August, September, October, and the succeeding April only, were made. The resulting regression coefficient is practically unchanged at -0.026% nitrogen for each additional inch of rain, and still statistically insignificant.

4. Discussion of Results

The negative correlation between yield and nitrogen content of grain is not wholly novel, similar results having been reported, for example, by Waldron (8) and by Malloch and Newton (3), though in these cases the relation was deduced from the analysis of different strains or plots grown in the same season. There is, moreover, a general impression that in years of high yield the nitrogen content tends to be low, and *vice versa*. Average seasonal differences of this nature must be largely the result of weather conditions. As pointed out by Finnell (1), when soil differences constitute an important factor, increased yield is not necessarily associated with a diminished nitrogen content.

The meteorological correlations of the preceding section, however, do not wholly agree with the conclusions (in part derived from the same data) of Shutt and Hamilton (6). These authors infer, from a comparison of the results secured over a period of years at two stations in eastern Canada (Charlottetown, P.E.I. and Kentville, N.S.) and two stations in western Canada (Scott, Sask. and Invermere, B.C.), that the "chief environmental factor conducive to high protein content" is "the drying out of the soil, due to scanty precipitation and high temperatures, during the later weeks of development and ripening of the kernel."

It may be noted that these conclusions refer to average climatic differences between widely separate regions, rather than to the usually less pronounced annual fluctuations in the weather conditions of one locality. Alsberg and Griffing (2) have emphasized this consideration, but as they themselves point out, climate and weather are "not from their very nature separable," and it seems reasonable to expect the direct effects of climatic differences to be simulated, on a smaller scale, by the vagaries of the weather. When considering the results of Shutt and Hamilton (6), however, the indirect effect of permanent climatic differences, in modifying soil conditions, should be borne in mind. It also seems desirable to point out (i) that the previous treatment of the experimental plots at Charlottetown and Kentville (including the growing of corn, oats, mangels, turnips, potatoes, clover hay, peas and hemp) was not identical with that at Invermere and Scott, and (ii) that in addition to the differences in the average rainfall and maximum temperatures for July and August between the eastern and western stations, referred to by them, the former also have a significantly higher average May and June rainfall.

On the other hand, the results deduced in Section 3 agree well with those reported by Russell and Bishop (5) as the outcome of a study of the influence of weather conditions on the nitrogen content of barley grown at Woburn, Beds., from 1885 to 1926. Here, also, the major influence of rainfall was found to be exerted in the first half of the growing season, and the effect of temperature at all times appeared to be slight. Russell and Bishop suggest that excessive spring rains may leach out nitrates, which would otherwise

remain to be utilized by the plant at a later stage of development. This may well be the explanation of their results, but it seems unlikely that such reasoning will apply with equal force under the semi-arid conditions of western Canada. Above-average rainfall may indeed even here carry the already available nitrates to somewhat lower levels and, by lowering the temperature of the soil, temporarily retard the rate of nitrification; but it seems likely that the main effect is produced in another way.

Other work by the author has shown a definite correlation between rainfall during the early part of the growing season and the subsequent yield of grain, under Canadian prairie conditions, and it is suggested that this is due to the stimulation of tillering and general vegetative development of the plant. Smith (7) in fact reports a correlation of +0.92 between rainfall and amount of tillering. As a result of this proliferation, the supply of available nitrogen must be distributed amongst an increased number of culms, and the total leaf area, devoted to the production of carbohydrates, will also be augmented, both circumstances tending towards a diminished proportion of nitrogen in the resulting grain. The insignificant effect of later rainfall may be due in part to the fact that (i) most of the plant nutrients derived from the soil are taken up before blossoming (2), and (ii) as pointed out by Russell and Bishop (5), the ratio of carbohydrates to nitrogen in the translocated material, is, even in an unfavorable season, so high that very large additional amounts of the former would have to be produced by the plant in order to affect appreciably the composition of the grain. Variation due to soil differences between the plots employed in different seasons may also have obscured the significance of real, though less pronounced, weather effects.

The apparently meagre effect of temperature during the later stages of development is perhaps surprising, for quite apart from any possible influence of desiccation on the proportionate translocation of carbohydrate and protein, higher temperatures might be expected to affect the ratio of nitrogenous to carbonaceous material in the developing grain by increasing the rate of respiration. It is of course possible that such respiratory effects did not attain the magnitude, relative to other sources of variation, necessary for detection. However, respiration is known to increase in an approximately exponential manner with temperature up to the lethal point, so that a difference of one degree has a greater effect at high than at low temperatures; the use of a linear average may therefore have failed to reveal the true effect of relatively short periods of very hot weather. From the point of view of the desiccation theory also, the inadequacy of temperature records, confined to daily maxima and minima, as an indication of the actual conditions, especially those resulting from hot dry winds, should not be overlooked. The systematic compilation of evaporation measurements at agricultural experiment stations would undoubtedly provide data of considerable value for use in future agricultural meteorological studies.

A high degree of correlation with the meteorological data was hardly to be expected of crop records secured from different small plots each season, in view of the effects upon both yield and composition of crops known to be occasioned by soil differences. Thus to cite only one instance, Newton and Malloch (4) report coefficients of variability ranging from 8.2 to 15.1 for the protein content of replicate plots of wheat varieties grown on the same field in the same season. As elsewhere suggested, however, such fluctuations will, to a considerable extent, cancel out when the annual results from any considerable area are considered, and a correspondingly closer relation between weather conditions and the average quality of the crop may be anticipated.

Acknowledgment

The author wishes to record his appreciation of the courtesy of Dr. F. T. Shutt, in making available for study the observations accumulated by him over a period of many years.

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EFFECT OF HEAT TREATMENT ON THE VISCOSITY OF GLUTEN DISPERSED IN ALKALI, ACID AND NEUTRAL SOLVENTS[1]

By W. H. Cook[2] and R. C. Rose[3]

Abstract

The viscosity of gluten dispersions in sodium hydroxide and acetic acid always decreased during heat treatment, the magnitude of the decrease being much larger in the former than in the latter solvent. Dispersions in urea solution decreased to a fixed viscosity level at temperatures below 70° C., but at higher temperatures the initial viscosity decrease of dilute dispersions was followed by an increase, then again by a decrease. At 60° C., dispersions in sodium salicylate increased in viscosity throughout the period of heat treatment, but at 80° C. the viscosity increased to a maximum and then fell off. This qualitative difference in the viscosity changes at temperatures above and below 70° C. in the neutral solvents, was the only evidence obtained to indicate that dispersed gluten undergoes any sudden change in character at a certain temperature, comparable to the so-called "coagulation point" of albumins. The results indicate that the changes occurring below 70° C. can be attributed mainly to the action of the solvent on the protein.

1. Introduction

There is considerable evidence to show that wheat and flour are affected by heat, certain treatments apparently improving the baking strength of the flour while others cause deterioration. Such treatments may affect several of the flour constituents, but it seems probable that the gluten proteins suffer the greatest change. Geddes (3, 4) has shown that the alteration in the proteins, due to heating, is evidenced by a decreased viscosity of leached, acidulated, flour-in-water suspensions, a decreased rate and extent of imbibition of the washed gluten, and a decrease in the ease of dispersion in neutral salt solutions. Herd (5) has also shown that the solubility of the proteins is decreased by severe heating, but the results of viscosity measurements on flour-in-water suspensions at pH 4.0–4.5 led him to the conclusion that severe heat treatment decreases the rate of swelling, but enhances the swelling power of the gluten.

The above evidence shows quite definitely that gluten protein in the solid state is rendered less soluble by heat treatment. This change has frequently been referred to as denaturation, but the exact nature of this phenomenon, even in such proteins as albumins which have been studied most extensively, is still obscure. It has been reported (1, 3, 4) that the extent of the change produced in gluten increases gradually with severity of heat treatment, although some of Geddes' (4) results suggest that over a certain range the

[1] *Manuscript received January 14, 1935.*

Contribution from the Division of Biology and Agriculture, National Research Laboratories, Ottawa, and the Department of Field Crops, University of Alberta, Edmonton, Canada. Published as paper No. 66 of the Associate Committee on Grain Research. This paper constitutes part of a thesis presented by the junior author to the University of Alberta, in partial fulfilment of the requirements for the degree of M.Sc.

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reaction has a high temperature coefficient similar to the known heat denaturation reaction of albumins. Results obtained with gluten and albumin, however, are not strictly comparable, since, in the investigation cited, the gluten protein was in a solid form, whereas albumin is generally studied in solution. It has also been shown (3, 4, 5) that the moisture content of the flour has a decided effect on the extent of the change which occurs on heating, and the quantitative relations may be complicated further by the presence of different electrolytes and of starch.

The lower solubility of the gluten protein after heat treatment has sometimes been attributed to coagulation, although the loss of solubility could be equally well explained by such a change as dehydration. It is, however, practically impossible to determine from experiments performed on flour or washed gluten whether or not coagulation does occur. On the other hand, gluten dispersed in the classical solvents, dilute acid and alkali, shows no evidence of visible coagulation on heating, although it is highly probable that these reagents prevent coagulation, or tend to redisperse any coagulum that might form as the result of heating. Cook (2) found that a 3% dispersion of gluten in 30% urea solution decreased in viscosity during heat treatment at 70° C., a behavior which suggests that heating favors either dehydration or dispersion rather than coagulation. Here again it is probable that the observed change was due to the effect of the solvent on the protein, rather than to the effect of heat alone.

The object of this investigation was to study the effect of heat on the viscosity of dispersed gluten with a view of determining whether there was any evidence of protein coagulation in any of the solvents. The authors (9) have already shown that the viscosity changes of gluten dispersions are determined, to a large extent, by the solvent employed, and as it doubtless affects the changes induced by heat treatment, the four solvents, dilute sodium hydroxide, dilute acetic acid, urea and sodium salicylate, were again used.

2. Methods

The flour, from which the gluten was obtained, and the method of preparing, analyzing and determining the viscosity of the dispersions were the same as those recently described (9). The dispersions were heat treated in stoppered tubes as described by Cook (2), the period of treatment being measured from the time the tubes were put in the bath until the sample was removed and placed in a water bath at 25° C. In some of the later experiments an improved method was employed in which portions of the dispersion were heat treated in small sealed tubes which were not opened until they had been cooled to 25° C. In this way the small amount of evaporation, which necessarily occurred when the larger tubes were opened for sampling, was avoided.

Urea solutions tend to decompose and during heat treatment they become alkaline in spite of added buffer substances. This necessitated frequent determinations of the hydrogen ion concentration to insure that the viscosity

measurements were obtained within the pH-stability range (9). As the viscosity is also affected by the urea concentration, an experiment was made to determine to what extent its decomposition might affect the results. It was found that heating a 30% urea solution for four hours at 80° C. resulted in an evolution of ammonia equivalent to about 0.5% of the urea nitrogen present. This would reduce the urea concentration only from 30 to 29.85% and it is evident (9, Fig. 2) that such a change would not affect the viscosity significantly.

The viscosity behavior of gluten dispersions during storage at 0 and 25° C. has been reported (9), and all the dispersions used in the present study were allowed to attain a reasonably constant viscosity before being heated. Initially, it was thought best to store the dispersions at 0° C. and, as urea solutions were employed first, all the dilute dispersions in this solvent, with the exception of the one that was treated when 30 days old, were stored at this temperature prior to heat treatment. All others were stored at 25° C. Dispersions in urea were heat treated at 40, 60, 70, 80, 90 and 100° C. but, in view of the results obtained, dispersions in the other solvents were treated at 60 and 80° C. only.

3. Results

Changes in Viscosity

The viscosity changes with time and temperature of heat treatment of gluten dispersions in all four solvents are shown in Figs. 1, 2 and 3. The concentration of the dispersions in mg. of protein nitrogen per gram of dispersion and the temperature of treatment are indicated in each figure. The number at the end of each curve gives the age of the dispersion in days, at the time heat treatment was started.

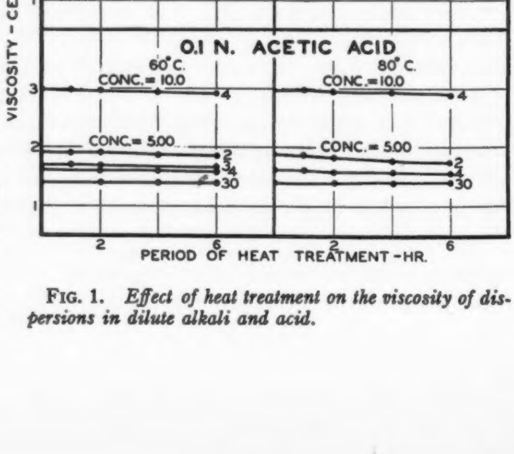

FIG. 1. *Effect of heat treatment on the viscosity of dispersions in dilute alkali and acid.*

The curves in Fig. 1 show that in all cases the viscosity of dispersions in sodium hydroxide decreased with time of heat treatment, the decrease being greater at 80° C. than at 60° C., and greater for concentrated than for

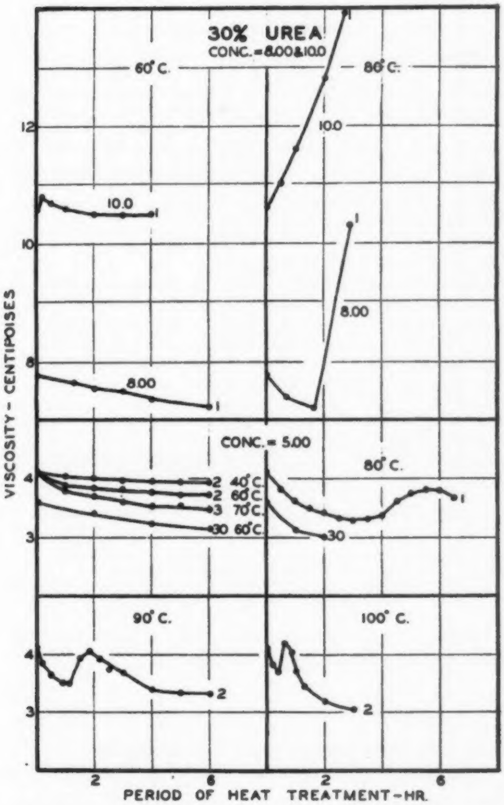

Fig. 2. *Effect of heat treatment on the viscosity of dispersions in urea solution.*

dilute dispersions. Of the latter, that which was eight days old when heat treated changed less than those which were only one or two days old. The viscosity of dispersions in acetic acid decreased slightly but showed much less change than in sodium hydroxide. The effect of heat treatment was practically the same in concentrated and dilute dispersions, and in dispersions of different ages.

It is evident from Fig. 2 that the viscosity of dispersions of 5.00 and 8.00 mg. of protein nitrogen per gram in urea solution decreased during heat treatment at temperatures of 70° C. or lower. At higher temperatures, and at 60° C. with the most concentrated dispersion, an increase in viscosity was observed at some stage of the heat treatment. The dilute dispersion that had been stored for 30 days at 25° C., prior to heat treatment, behaved in essentially the same way as the one that had been stored for two days at 0° C.

The results plotted in Fig. 3 show that the viscosity of dispersions in sodium salicylate increased throughout the entire period of heat treatment at 60° C., but at 80° C. it increased to a maximum and then decreased. At both temperatures the changes were greater in the concentrated dispersions. Here again the dispersion that had been stored for 30 days behaved in a similar manner to those that had been stored for two days.

Changes in pH

During the heat treatment the changes in the hydrogen ion concentration of all dispersions were followed and the initial and final values are given in Table I. The duration of treatment was usually six hours, but with certain

dispersions in urea solution it was shorter, as shown by the curves in Fig. 2. The pH of dispersions in sodium hydroxide decreased during heat treatment, the decrease being greater at the higher temperature and in the more concentrated dispersion. In dispersions in acetic acid and sodium salicylate no significant change in pH occurred, whereas in urea solution the pH increased, a result which can be attributed entirely to the decomposition of urea. As it has already been shown (9) that the viscosity is affected by the pH when it exceeds 9.2, no viscosities of dispersions in urea solution have been reported where the pH exceeded this value.

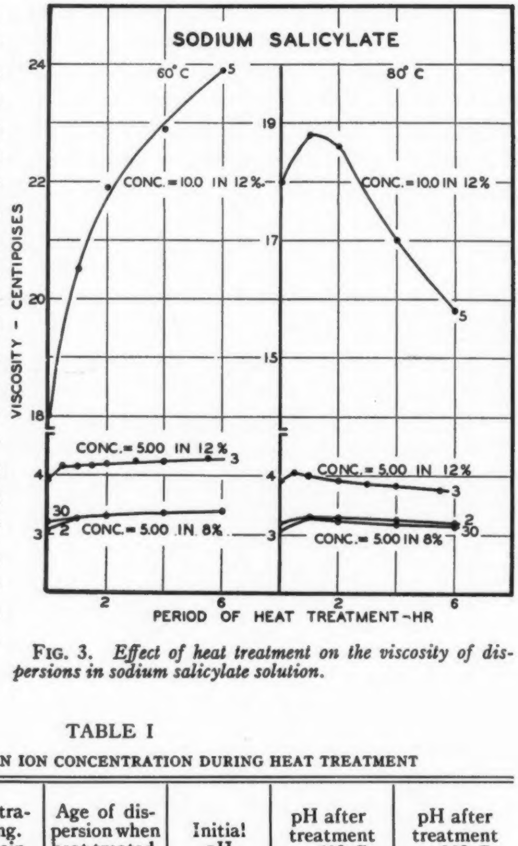

FIG. 3. *Effect of heat treatment on the viscosity of dispersions in sodium salicylate solution.*

TABLE I

CHANGES IN HYDROGEN ION CONCENTRATION DURING HEAT TREATMENT

Solvent	Concentration, mg. of protein N per gm.	Age of dispersion when heat treated, days	Initial pH	pH after treatment at 60° C.	pH after treatment at 80° C.
0.1 *N* Sodium hydroxide	5.00	1	12.57	12.30	11.84
	5.00	2	12.50	12.30	11.81
	5.00	8	12.00	11.92	11.20
	10.00	1	12.24	11.51	10.50
0.1 *N* Acetic acid	5.00	2	3.75	3.70	3.73
	5.00	3	3.72	3.54	—
	5.00	4	3.74	3.77	3.81
	5.00	30	3.77	3.76	3.75
	10.00	4	4.02	4.06	4.05
30% Buffered urea	5.00	2	6.82	7.21	8.74*
	5.00	30	7.55	7.73	8.05
	8.00	1	6.87	7.16	7.88
	10.00	1	6.79	6.95	7.72
8% Sodium salicylate	5.00	2	6.79	6.76	—
12% Sodium salicylate	5.00	3	6.90	6.86	6.89
	10.00	5	6.82	6.82	6.83

Dispersion one day old.

Salting-out Tests

A number of salting-out tests were made on dilute dispersions in the neutral solvents after various heat treatments. The results are given in Table II. Heat treatment of dispersions in urea solution at 40° C. had little effect on the amount salted out, but diminished it if anything, whereas heating at 70 and 80° C. increased the amount precipitated. Heating at 60° C. had no effect on dispersions in 8% sodium salicylate but after heat treatment at 80° C. no precipitate was obtained.

TABLE II

PROTEIN NITROGEN SALTED-OUT BY MAGNESIUM SULPHATE FROM DILUTE DISPERSIONS AFTER VARIOUS HEAT TREATMENTS

Solvent	Heat treatment	Viscosity, centi-poises	$MgSO_4$ added to 10.0 ml.	Protein nitrogen precipitated	
				mg.	%
30% Urea	Nil	3.99	2.75 ml. of 20%	14.0, 16.8	28.0
	9.5 hr. at 40° C.	3.91	2.75 ml. of 20%	13.8, 11.2	22.7
	2 hr. at 70° C.	3.58	2.75 ml. of 20%	23.5, 23.7	42.9
	1 hr. at 80° C.	3.51	2.75 ml. of 20%	26.2, 26.1	47.5
8% Sodium salicylate	Nil	3.09	1.50 ml. of 5%	12.5, 12.2	22.6
	6 hr. at 60° C.	3.41	1.50 ml. of 5%	12.9, —	23.5
	6 hr. at 80° C.	3.21	1.50 ml. of 5%	0.0, 0.0	0.0

Stability of Viscosity Following Heat Treatment

A number of dispersions in urea solution were stored at 0° C. after being heat treated, and the viscosity determined periodically. The results, given in Table III, show that the viscosity remained constant during storage. This study did not include any dispersions that had increased in viscosity during heat treatment, so no information was obtained as to the stability of these systems.

TABLE III

VISCOSITY OF DILUTE DISPERSIONS IN 30% UREA SOLUTIONS STORED AT 0° C. AFTER HEAT TREATMENT

Heat treatment	Duration of storage at 0° C., days	Vis-cosity, centi-poises	pH	Heat treatment	Duration of of storage at 0° C., days	Vis-cosity, centi-poises	pH
9.5 hr. at 40° C.	0	3.91	6.88	8 hr. at 70° C.	0	3.17	7.99
	1	3.92			0.2	3.13	
	3	3.94			1	3.10	
	10	3.90	6.87		2	3.11	
2 hr. at 70° C.	0	3.58	7.11		8	3.13	7.89
	1	3.55		1 hr. at 80° C.	0	3.51	7.20
	3	3.56			1	3.49	
	10	3.55	7.10		3	3.49	
4 hr. at 70° C.	0	3.38	7.44		10	3.50	7.35
	0.4	3.37					
	1	3.39					
	2	3.39					
	8	3.39	7.49				

Order of the Reaction

An attempt was made to determine the order of the reaction causing the viscosity decrease by calculating the time required for one-half of the viscosity decrease in dispersions of different concentrations. Since dispersions could be prepared and centrifuged at 25° C. only, the data obtained from experiments conducted at this temperature were employed (9, Fig. 5). Even at this temperature, the viscosity changes in sodium hydroxide and sodium salicylate were too small to permit a reliable estimation of the order of the reaction in these solvents. The results obtained with dispersions in acetic acid and urea, given in Table IV, show that the time required for half the viscosity fall-back was independent of the initial concentration. The reaction is therefore unimolecular.

TABLE IV

ORDER OF REACTION CAUSING VISCOSITY DECREASE

Solvent	Concentration, mg. of protein N per gm.	Initial viscosity, centipoises	Final viscosity,* centipoises	Time required for one-half of viscosity decrease, hr.	Reaction
0.1 *N* Acetic acid	5.10 5.46 10.3	3.68 4.13 8.70	1.56 1.62 2.90	10.0 10.0 10.0	Unimolecular
30% Urea	5.24 9.48 10.7	4.70 12.4 15.0	3.84 11.0 12.4	4.0 3.5 4.0	Unimolecular

**Final viscosity in acetic acid taken when dispersions six days old; final viscosity in first two dispersions in urea taken when they were two days old, and in last dispersion when 1.2 days old.*

4. Discussion

In the experiments of this type, the changes observed during heat treatment are due, not only to the action of heat, but also to the action of the solvent on the protein. It seems probable that, wherever the changes observed at the higher temperatures differed only quantitatively from those observed at 0 or 25° C., they were due to the action of the solvent. Qualitative differences, however, may reasonably be attributed to the action of heat.

Employing this criterion, the viscosity changes observed during the heat treatment of dispersions in alkali and acid can be attributed entirely to the action of the solvent. The pH decrease observed during the heat treatment of dispersions in sodium hydroxide indicates the exposure of more base-binding groups, and if Wu and Chen's (10) hypothesis is correct these results suggest that denaturation took place without coagulation.

In urea and sodium salicylate solutions the changes induced by heat treatment at temperatures below 70° C. can also be attributed to the action of the solvent, heat merely accelerating the reaction observed at lower temperatures.

The viscosity of dispersions in sodium salicylate increased more rapidly on heat treatment at 60° C. than during storage at 25° C. (9), and in concentrated dispersions the high viscosity attained at both temperatures suggests that some form of coagulation took place. The salting-out tests conducted on heat treated dispersions in this reagent are of little help in interpreting the changes in viscosity, but they lend support to the view that the reactions occurring at 60° C. and 80° C. are qualitatively different (Table II).

Dilute dispersions in urea solution were exposed to several temperatures below 70° C., and it is evident from the results (Fig. 2) that the final viscosity is apparently dependent on the temperature. A further study of this relation was made by computing the specific hydrodynamic volume of 1 mg. of protein nitrogen by means of Kunitz's (6) equation and plotting the values against the temperature of treatment. At temperatures of 40, 60 and 70° C., the final viscosity at the end of the heat treatment was used in the computation, and at 25° C. the value after storage for 20 hr. (9, Fig. 5) was used. As dispersion in urea could not be effected at 0° C., the probable specific hydrodynamic volume after dispersion and storage at this temperature was obtained by extrapolating from the constant values obtained during storage at 0° C., after initial periods of 2, 4, 6, 8, and 12 hr. at 25° C. (9, Fig. 4). The results are given in Fig. 4 along with the specific hydrodynamic volume when the viscosity was at a minimum, and at a maximum, at temperatures above 70° C. The fact that the values of the specific hydrodynamic volume at all temperatures up to and including 60° C. lie on a smooth curve is taken as evidence that the viscosity decrease observed at these temperatures is due to a continuation of the dispersing action of the solvent.

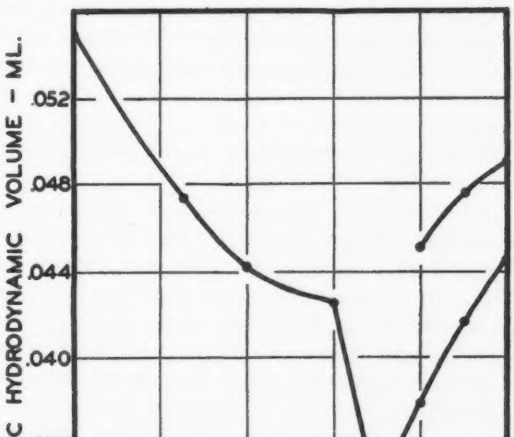

FIG. 4. *Effect of temperature on the specific hydrodynamic volume of 1 mg. of protein nitrogen in dilute dispersions in urea.*

This conclusion is supported by the fact that gluten dispersions were less easily salted-out after storage at 25° C. (9) and heat treatment at 40° C. (Table II). This behavior also indicates that the decrease in viscosity which occurred under these conditions was due to a further dispersion of the gluten rather than to dehydration, for, had the latter occurred the protein would have been more easily salted-out.

It has already been shown that the reaction causing the viscosity decrease at 25° C. in urea solutions is unimolecular, and doubtless the dispersion reaction at all temperatures below 70° C. is of the same order.

Even at temperatures above 70° C., where the effect of heat is most pronounced, it is evident from Figs. 2 and 3 that the solvent has a decided effect on the nature of the viscosity changes. Thus the viscosity of dispersions in sodium salicylate at first increased and then decreased, while that of dispersions in urea solution decreased, then increased to a maximum, and finally decreased again. The quantitative differences in the viscosity changes above and below 70° C. are not as pronounced with dispersions in sodium salicylate as in urea solution. The initial viscosity increase observed in the former solvent at 80° C. is interpreted as indicating a continuation of the same reaction which occurred at lower temperatures, while the final viscosity decrease suggests a further dispersion of the original material or a redispersion of the aggregates formed during the initial period.

Regarding the nature of the changes which occur in dilute dispersions in urea solution at temperatures of 70° C., or higher, it is evident that the specific hydrodynamic volume is at a minimum at 70° C. (Fig. 4), and from this it is concluded that dehydration takes place prior to the coagulation reaction, which causes the viscosity increase observed at higher temperatures. This conclusion is supported by the results of salting-out tests which show that the protein is more easily precipitated from dispersions heated for 1 hr. at 70° C. or 80° C. than from dispersions held at lower temperatures for the same period. As no increase in viscosity occurred under these conditions, the increased instability of the dispersions must be attributed to dehydration rather than coagulation.

The viscosity increase in dilute dispersions in urea solution which follows the initial decrease indicates that after dehydration reached a definite stage, coagulation set in. The viscosity decrease subsequent to the rise may be due to a redispersion of the coagulum and it seems likely that the several reactions proceed to some extent simultaneously. The period required for the viscosity to reach either a minimum or a maximum decreased as the temperature increased. Thus a maximum viscosity was obtained in about 5.5 hr. at 80° C., 1.8 hr. at 90° C. and 0.6 hr. at 100° C. As each additional 10° C. rise in temperature decreased the time required to reach a maximum to about one-third of its former value, the Q_{10} for these reactions is evidently about 3. The interval required for the dispersion to attain a maximum viscosity was used to calculate the critical increment of the process. This was found to be 177,000 cal. Lewis found the critical increment for the heat denaturation of haemoglobin to be 77,500 cal. (7) and of egg albumin 130,000 cal. (8). Considering the magnitude of these values and the relatively large difference between the values for albumin and haemoglobin, the above value for gluten is not at all improbable.

Considering all the results there appear to be four reactions which contribute to the viscosity changes, namely, dispersion, dehydration, coagulation and

redispersion, and it is possible that secondary changes such as hydrolysis also play a role. In alkali and acid there is no evidence of coagulation, and from the present results it is impossible to determine the number of reactions affecting the viscosity. In concentrated dispersions in sodium salicylate the viscosity increase is sufficiently marked to suggest coagulation, and this reaction may also cause the viscosity increase observed in dilute dispersions in this same reagent. At 80° C., coagulation is apparently followed by redispersion. In dilute dispersions in urea solution the dispersion reaction predominates at temperatures up to and including 60° C. At higher temperatures dehydration apparently sets in and it is followed by coagulation, the final reaction being a redispersion of the coagulum. With concentrated dispersions in this reagent coagulation may occur with no previous indication of dehydration.

The conclusion that gluten undergoes the greatest change at temperatures of 70° C. or higher is in agreement with the results of baking tests and viscosity measurements reported by Geddes (4). These show that flour heated for eight hours at 155° F. (68.3° C.) was not altered nearly as much as flour heated for a similar period at 160° F. (71.1° C.). It appears therefore, that gluten dispersed in the neutral solvents, particularly urea solution, is affected by heat in the same way as flour. Since this is not true of dispersions in alkali or acid, it is concluded that these solvents alter the protein to such an extent that it no longer exhibits the properties of native gluten.

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HYDROLYSIS OF GLUTEN INDUCED BY THE SOLVENT[1]

By W. H. Cook[2] and R. C. Rose[3]

Abstract

Dilute sodium hydroxide and dilute acetic acid both hydrolyze gluten even at 0° C., the reaction constant and temperature coefficient being greatest in dispersions in alkali. The reaction appears to be unimolecular in both reagents although it seems probable that neither of these can effect complete hydrolysis. No appreciable hydrolysis occurred in gluten dispersed in urea or sodium salicylate solutions, even after relatively drastic heat treatment.

1. Introduction

A study has been made of the relative extent to which dispersed gluten is hydrolyzed by different solvents during storage at various temperatures. Four dispersing agents were employed, namely, 0.1 *N* sodium hydroxide, 0.1 *N* acetic acid, 30% urea and 8% sodium salicylate, representing the solvents that are known to be capable of dispersing gluten completely. There is considerable evidence that dilute alkali and acid, in addition to causing denaturation, also induce secondary changes such as hydrolysis in proteins. Thus dilute alkali is known to cause decomposition of albumin (6), haemoglobin (8) and gluten (1); and acid, although its action may be less drastic, decomposes edestin (7) and other proteins (8). The neutral solvents may also denature proteins but experiments already reported (5) indicate that they alter the original properties of gluten less than dilute alkali and acid. The main object of this study was to determine which of the gluten solvents caused least hydrolysis, and also to see whether this secondary change was related to the alterations in viscosity already reported (2, 5).

2. Experimental

Methods

Dispersions containing 5 mg. of protein nitrogen per gram were prepared as described by Rose and Cook (5), subjected to various treatments, and an attempt was then made to determine, by precipitation methods, the proportion of the protein that had been hydrolyzed. Both tannic and trichloracetic acid were used as precipitating reagents for all dispersions excepting those in urea, where tannic acid only was used since trichloracetic acid had proved unsatisfactory (5). Hiller and Van Slyke (3) have shown that 2.5% trichloracetic acid is suitable for separating proteins from their split products, while Lundin and Schröderheim (4) have shown that tannic acid precipitates some peptones in addition to proteins.

[1] *Manuscript received January 14, 1935.*
Contribution from the Division of Biology and Agriculture, National Research Laboratories, Ottawa, and the Department of Field Crops, University of Alberta, Edmonton, Canada. Published as paper No. 67 of the Associate Committee on Grain Research.

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Dispersions in urea were analyzed as previously described (5), the precipitated nitrogen being determined directly. With the other solvents the nitrogen content of the filtrate was determined in the initial experiments, but later it was found that with aged dispersions in sodium hydroxide the odor of ammonia was evident, and here again it was considered more accurate to determine the nitrogen content of the precipitate. The weight delivered by a 20 ml. pipette was determined and this amount of dispersion added to 180 ml. of a solution containing sufficient precipitating reagent to make the final concentration 2.5% trichloracetic acid or 2.0% tannic acid. Precipitation with tannic acid left a clear supernatant liquid which was filtered directly into a Kjeldahl flask. As the supernatant liquid from the trichloracetic acid precipitations was cloudy, it was centrifuged and decanted, then brought to a boil and allowed to stand overnight at room temperature before filtering. This procedure yielded a clear filtrate and it is unlikely that it caused any significant hydrolysis, since Hiller and Van Slyke (3) found that boiling for 15 min. had no measurable effect.

The addition of the acidic precipitating reagents always caused a precipitation of salicylic acid from dispersions in sodium salicylate, and it was at first thought that this might interfere with the quantitative separation of the protein and non-protein nitrogen. In early attempts to overcome this difficulty, sufficient alcohol was added to keep the salicylic acid in solution, but it was found that the protein could not then be precipitated quantitatively from these solutions, and neither filtering nor centrifuging would render the supernatant liquid clear. An effort was then made to determine whether the precipitation of salicylic acid actually had any effect on the results. A dispersion in sodium hydroxide containing 5 mg. of nitrogen per gram was analyzed, and it was found that 74% of this nitrogen was precipitated by tannic acid. Ten grams of sodium salicylate was then added to 90 ml. and the dispersion analyzed as before. It was found that 76% of the nitrogen was precipitated. These results indicated that the precipitation of salicylic acid along with the protein had little effect, and no attempt was made to prevent its precipitation in subsequent experiments.

Results

The amount of nitrogen not precipitated by tannic acid and trichloracetic acid from gluten dispersions in sodium hydroxide, acetic acid and sodium salicylate, after various storage and heat treatments, is reported in Table I. The results obtained with dispersions in urea solution are given separately in Table II, owing to the different method by which these were obtained. These show definitely that under similar conditions, the amount of gluten hydrolyzed by the different dispersing agents decreases in the order, sodium hydroxide, acetic acid, and the neutral solvents, there being no significant hydrolysis by either of the last-named. In urea solution the results indicate that the original analysis was about 1% too low, while the slight progressive increase in the protein concentration following heat treatment can probably be attributed to evaporation or experimental error. In this experiment the

nitrogen content of the filtrate from dispersions in sodium hydroxide was determined, and as a loss of ammonia may have occurred these results are possibly somewhat low. Tannic acid always precipitated more nitrogen than trichloracetic acid, as would be expected, since the former reagent is known to precipitate such degradation products as peptones while the latter precipitates only proteins (3, 4). The difference between the amount of nitrogen precipitated by the two reagents suggests that the hydrolysis is a general disintegration of the protein molecule, rather than the splitting off of terminal amino acids or amide groups, for, had the latter occurred the amount of nitrogen precipitated by each of the reagents would have been approximately the same. The amount of nitrogen not precipitated by tannic acid is about 30% of that not precipitated by trichloracetic acid from dispersions in alkali and about 40% from dispersions in acid (Table I). This suggests that the degradation effected by dilute alkali differs from that caused by dilute acid.

TABLE I
HYDROLYSIS OF DISPERSED GLUTEN

Treatment	Solvent	Percentage of total nitrogen not precipitated	
		By tannic acid	By trichloracetic acid
Stored for 6 hr. at 25° C.	0.1 *N* Sodium hydroxide 0.1 *N* Acetic acid	1.9 1.4	7.0 3.4
Stored for 7 hr. at 25° C.	8% Sodium salicylate	0.8	—
Stored for 8 days at 25° C.	0.1 *N* Sodium hydroxide	12.9	30.9
Stored for 30 days at 25° C.	0.1 *N* Acetic acid 8% Sodium salicylate	9.0 1.0	31.0 1.1
Stored for 21 days at 0° C.*	0.1 *N* Sodium hydroxide	6.8	17.1
Stored for 36 days at 0° C.*	0.1 *N* Acetic acid	3.5	11.2
Treated at 60° C. for 6 hr. after 2 days at 25° C.	0.1 *N* Sodium hydroxide 0.1 *N* Acetic acid 8% Sodium salicylate	11.6 3.4 0.8	37.0 10.8 1.0
Treated at 80° C. for 6 hr. after 2 days at 25° C.	0.1 *N* Sodium hydroxide 0.1 *N* Acetic acid 8% Sodium salicylate	22.0 4.0 0.9	54.0 11.6 1.3
Treated at 60° C. for 6 hr. after 8 days at 25° C.	0.1 *N* Sodium hydroxide	16.2	41.8
Treated at 60° C. for 6 hr. after 30 days at 25° C.	0.1 *N* Acetic acid 8% Sodium salicylate	9.7 1.0	32.8 1.0
Treated at 80° C. for 6 hr. after 8 days at 25° C.	0.1 *N* Sodium hydroxide	21.2	51.9
Treated at 80° C. for 6 hr. after 30 days at 25° C.	0.1 *N* Acetic acid 8% Sodium salicylate	9.6 1.2	33.4 1.4

**An initial period of eight hours at 25°C. preceded the storage at 0° C.*

TABLE II

RECOVERY OF GLUTEN FROM DISPERSIONS IN 30% UREA SOLUTION

Treatment of dispersion	Percentage recovery
Stored for 30 days at 25° C.	101.0
Treated at 60° C. for 6 hr. after 2 days at 25° C.	101.0
Treated at 60° C. for 3 hr. after 30 days at 25° C.	101.3
Treated at 80° C. for 1 hr. after 30 days at 25° C.	100.7
Treated at 80° C. for 2 hr. after 30 days at 25° C.	101.5
Treated at 80° C. for 3 hr. after 30 days at 25° C.	102.0

Another experiment was performed in an attempt to determine the order of the hydrolysis reaction induced by dilute alkali and acid. Trichloracetic acid was the only precipitating reagent employed and the precipitate from dispersions in sodium hydroxide was analyzed directly in order to avoid the error due to the possible loss of ammonia. The results obtained after heating for various periods at 30 and 80° C. are given in Table III. These dispersions were only four hours old when the first determination was made, and it is evident that appreciable hydrolysis had occurred at this time.

TABLE III

RATES OF HYDROLYSIS

Solvent	Temperature of treatment, °C.	Duration of treatment, hr.	Concentration, percentage recovery	$K \times 10^6$ (unimolecular)
0.1 *N* Sodium hydroxide	30	0	88.0	
		50	72.4	
		122	62.4	1.7
0.1 *N* Sodium hydroxide	80	0	88.0	
		1.1	53.6	
		2	46.4	
		3	38.8	
		6	39.5	
		9	27.8	
		18	26.8	150
0.1 *N* Acetic acid	30	0	93.0	
		50	86.3	
		122	75.2	
		199	71.0	
		336	64.4	0.55
0.1 *N* Acetic acid	80	0	93.0	
		9	88.7	
		18	87.6	
		27	85.4	
		50	81.0	1.2

Neither the logarithm nor the reciprocal of the protein concentration gave a straight line when plotted against time, from which it appeared that the reaction was neither unimolecular nor bimolecular. It can be seen from the table, however, that at 80° C. the hydrolysis reaction in sodium hydroxide was apparently complete in nine hours, at which time about 27% of the original

nitrogen was still in the form of protein. It appears therefore that dilute sodium hydroxide is not capable of carrying the reaction to completion. In consequence the logarithm of the quantity (C-27), where C is the concentration given in Table III, was plotted against time. This gave a linear relation between the two quantities at both 30 and 80° C., indicating that the reaction was unimolecular. Unfortunately, the experiment with dispersions in acetic acid was not extended over sufficient time to obtain the limiting value, but when the logarithm of the quantity (C-27), as defined above, was plotted against time, the relation was practically linear. Since the reactions are apparently unimolecular, the appropriate reaction constants were calculated and are reported in Table III. From these it is evident that the hydrolysis of dispersions in sodium hydroxide is more rapid, and is accelerated more by a rise in temperature than that of dispersions in acetic acid. The results given in Table I confirm this conclusion.

3. Discussion

These findings are, to some extent, in agreement with the results of viscosity measurements (2), the decrease in viscosity during heat treatment being much greater in sodium hydroxide than in acetic acid. The extent of hydrolysis does not parallel the decrease in viscosity in all cases, however, as the viscosity of dispersions in sodium hydroxide did not change significantly during storage at 25° C., although quite extensive hydrolysis occurs under these conditions. Dispersions in acetic acid showed the reverse behavior, *i.e.*, a decrease in viscosity but comparatively little hydrolysis. If the latter is the only factor affecting the dispersions after several days storage, the viscosity behavior might be explained by assuming that the complexity of the resulting degradation products differed in the two solvents.

Since the present study indicates that hydrolysis does not occur in the neutral solvents, it is doubtful whether the viscosity changes previously observed (2, 5) can be attributed to this effect. Those viscosity decreases must, therefore, have been due to dispersion or dehydration.

It has already been shown (2, 5) that gluten dispersed in the neutral solvents retains the properties of the native protein to a higher degree than do dispersions in dilute alkali or acid. Whether this change brought about by the latter solvents is due to a primary (dispersion and denaturation) or to a secondary (hydrolysis) alteration of the protein is not known, but it is evident from the present results that drastic secondary changes occur in both alkali and acid in a remarkably short time.

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THE TREATMENT OF HYDRATED LIME WITH ALUMINIUM SULPHATE[1]

By A. F. Gill[2] and T. H. Way[3]

Abstract

This paper deals with the question of "workability" of hydrated limes for finishing purposes. The authors subscribe to the "colloid" conception of the mechanism underlying the well known superiority of undried putties over dry hydrates, on the one hand, and of magnesian or dolomitic hydrates over those high in calcium, on the other.

A description is given of an investigation made to determine whether an artificial gel could be applied to particles otherwise of poor quality by allowing them to react with aluminium sulphate. It was anticipated that the calcium hydroxide would react to form calcium sulphate and give a precipitate of aluminium hydroxide which, being relatively insoluble in the alkaline medium, would tend to coat the particles of calcium sulphate and residual calcium hydroxide. The effect of additions under different conditions was observed quantitatively by means of a flow-table plasticimeter. Favorable laboratory results were followed by practical trials which successfully demonstrated the usefulness of this treatment.

The value of hydrated lime for use as finishing plaster depends in large measure on that property, variously called "plasticity," "workability" or "fatness," which allows it to spread readily under the trowel, without "tearing" or "rolling", to give a smooth uniform surface. In this respect magnesian or dolomitic hydrates are usually superior to those high in calcium although the latter, other things being equal, are superior when slaked with an excess of water and used without being dried. The possibility of improving the properties of hydrated high-calcium lime to render it as valuable as dolomitic material for plaster finishing has long been of interest to owners of small lime plants, and indeed to those of many larger ones to whom dolomitic stone is not readily available.

The authors subscribe to the view that workability of a lime depends upon the presence in it of a major proportion of particles of colloidal size surrounded by a lubricating layer of adsorbed water (7). Such a conception serves to explain various physical and mechanical phenomena characteristic of desirable limes, such as rate of settlement from water suspension, "volume in water," and the important factor of water retention when applied to porous backings.

The fact that a hydrate, once dried, cannot be greatly improved by aging in water points to the view that the particles function as an irreversible colloid. That being the case, it is not surprising that no radical improvement in the quality of dry hydrates is obtainable through changes in methods of preparation.

[1] *Manuscript received December 26, 1934.*
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Attempts have been made by various investigators to improve hydrated lime through the addition of chemicals to the limestone (3), to the quicklime (4) or to the dry hydrate (2). Though certain improvements have been reported, no treatment of this kind, as far as the authors are aware, is being used commercially.

Consideration of this matter led to the opinion that the best possibility lay in an addition to the hydrated lime, subsequent to its preparation, of a reagent which would affect its properties in a mechanical way. It was recognized that additions of such materials as diatomite, bentonite, etc., had not yielded any great deal of success. Some success was attained in this laboratory by the use of crysotile asbestos fibre of "floats" grade. This material is very absorptive of water and, what is probably more important, has a rough fibre surface which would tend to hold lime particles apart. The chief disadvantage in its use lies in the fact that it is generally of a slate gray color and would therefore seriously detract from the salability of any hydrate with which it was mixed.

It was considered that an ideal admixture would be a material which would precipitate an artificial "gel" around the lime particles. This suggested the possibility of using aluminium sulphate. It was recognized that the calcium hydroxide would react with this material to precipitate calcium sulphate and gelatinous aluminium hydroxide. The latter appeared to have the specific property desired, while the calcium sulphate, being chemically identical with the gypsum commonly used with hydrated lime as gauging plaster, would do no harm. In addition, the products of this reaction are white, and thus would have no deleterious effect on the color of the product.

Qualitative trials made in the laboratory indicated that the proposal was technically feasible. It appeared, however, that an amount approaching 10% of the weight of a high-calcium hydrate would be necessary in many cases to give sufficient improvement. For this reason, and because information had been obtained that aluminium sulphate had been used by other investigators, generally with the object of increasing the strengths (1, 5, 6, 8), the matter was dropped for some time. Owing, however, to the receipt of repeated inquiries by this laboratory from manufacturers interested in the improvement of their product and a considerable reduction in the market price of aluminium sulphate, the matter was re-opened, and accurate experimental results were obtained.

Methods of Measuring Plasticity

Numerous efforts have been made to develop standard tests for plasticity or workability of hydrated lime; perhaps the best known is the Emley instrument which is much used in the United States. In the determination by means of this instrument, the "suction" feature of the backing predominates. The "working time," the period during which the putty remains coherent while it is being sheared on a standard porous backing, is measured. A disadvantage of this instrument from the point of view of development work lies in the fact

that considerable care is necessary in the preparation of the backings and a limited number of tests can be put through in the course of a working day. Another instrument which has considerable interest is a flow-table device developed by Mr. J. S. Cowper, Building Research Station, Carston, England. This apparatus is very simple in its action and was found to give such satisfactory results that it was used throughout the investigation reported here. It depends for its action on the spreading of a cone of putty when "knocked" on the platform of a standard flow table under specified conditions. The more plastic a putty the greater number of knocks required to spread it to a specified diameter. This very interesting characteristic presumably depends on the voids existing in the mass, coupled with the "cushioning" or shock-absorbing effect of the gelatinous surface layers.

For detailed specifications of the flow table the reader is referred to publications of the Building Research Station, Department of Scientific and Industrial Research, England.

The relation between the results obtained with the flow table and those obtained with the Emley instrument is not a linear one, but the following indicates the approximate degree to which the flow table shows the properties of a lime: poorly plastic limes, 12 to 14 knocks; limes of good quality for finishing purposes, 20 to 30 knocks; very highly plastic limes, more than 30 knocks. Limes requiring more than 30 knocks have a tendency to slump and, when in place, to remain soft for undesirably long periods, thus slowing up the finishing operation.

Major changes in the plasticity of dolomitic or magnesian hydrates as indicated by this instrument take place when the material is aged in water. It is rather difficult to explain this satisfactorily, but in one case a relative workability at 24 hr., corresponding to 40 knocks, was reduced to about 21 knocks on aging 120 hr. out of contact with air. (Fig. 1 shows graphically this decrease.) It was therefore necessary to observe carefully the time factor in aging, particularly in the testing of magnesian hydrates. For the tests reported below, an aging period of 17 hr. was adopted as standard.

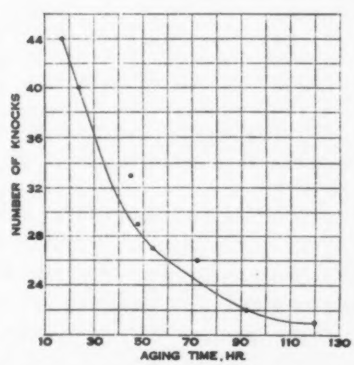


FIG. 1. *Effect of aging time on plasticity; lime No. 4598 (dolomitic). The two points that lie appreciably off the curve were obtained when a large excess of tempering water was used.*

Experimental

As it was considered that appreciable proportions of aluminium sulphate would be necessary to give sufficient improvement in the properties of numerous limes, a comparison was made of the color given to a white lime by ordinary

commercial aluminium sulphate, $Al_2(SO_4)_3.18H_2O$, and that given by "iron-free" quality when both were mixed with nine parts of hydrated lime and tempered with water. No difference in color could be discerned, and it was concluded that the cheaper product could be used successfully. This material is available in large quantities for purposes of water purification and is usually purchased according to the following specification:

Not less than 17% water soluble alumina.
Not more than 0.5% total water insoluble material.
Not more than 0.75% Fe_2O_3.

The material is obtainable in the granular form.

In the preliminary trials of aluminium sulphate additions, the limes used were of high-calcium content, as shown by the chemical analyses given in Table I.

TABLE I

ANALYSES OF LIME SAMPLES

Lab. no. of sample	SiO_2	R_2O_3	CaO	MgO	H_2O	CO_2
4597	3.8	1.4	71.8	Trace	22.2	0.8
7300	1.2	0.6	72.0	1.3	23.7	1.3
7301	0.6	0.5	73.0	0.7	23.5	1.9

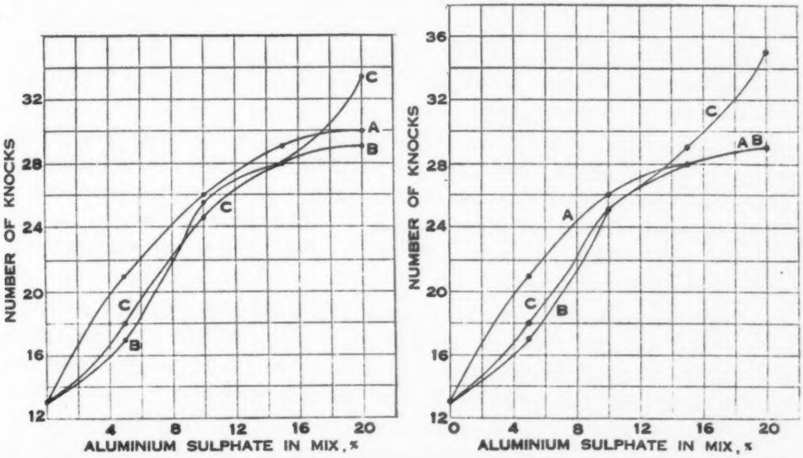
When tempered with water, aged, and brought to standard consistency, each of these samples required 13 knocks in the plasticity test, and was definitely inferior to finishing hydrate of acceptable quality. In treating them with aluminium sulphate the initial procedure followed was to dissolve a sufficient amount of the salt in rather less than the amount of water required for tempering, add this to the dry material and bring to the standard consistency with distilled water.

Figs. 2 and 3, Curve A, show quantitatively the effect of this addition; the results agree within one knock for the different limes. It will be observed that an aluminium sulphate content of 5–20% gives a lime equivalent in knock requirements to finishing hydrates of good quality.

As the only practical method of treating hydrated lime with aluminium sulphate would be to add it in the pulverized form, tests were made to determine the influence on the resulting plasticity of dry mixing of the aluminium sulphate. It was recognized that the material would have to be reasonably finely ground and no tests were made on sulphate coarser than 80 mesh.

Commercial aluminium sulphate is rather a difficult material to pulverize because it tends to decompose at higher temperatures and dissolve in its water of crystallization. This causes it to "gum" in pebble mills or disc pulverizers. Although no experiments have been made in this connection, there does not seem to be any reason why the sulphate could not be ground

successfully in an impact pulverizer, provided reasonable care were taken to avoid overheating. Furthermore, grinding in the presence of the required amount of lime gives ample protection against "gumming" or "balling." Tests were accordingly made of the effect on the properties of limes Nos. 4597, 7300, and 7301 of additions of aluminium sulphate both when ground separately to minus 80 mesh, and when mixed with the lime and ground to minus 100 mesh.



FIGS. 2 and 3. *Effect of addition of aluminium sulphate ($Al_2(SO_4)_3 . 18H_2O$) on plasticity.* FIG. 2. *Lime No. 7300 plus lime No. 7301, average of two determinations.* FIG. 3. *Lime No. 4597. In both figures—A, lime plus sulphate in solution; B, lime plus sulphate of minus 80 mesh; C, mixture of lime and sulphate ground to minus 100 mesh.*

Curves B and C, Figs. 2 and 3, show these results. They may be compared with those obtained by adding the aluminium sulphate in solution. It will be observed that the effect is comparable in all cases except when the lime and aluminium sulphate were pulverized together. In this instance the continued improvement in knock requirements is rather difficult to explain. The results, however, were duplicated in a number of tests. It is evident that for small amounts of aluminium sulphate, granted good mixing conditions, the best results are given when it is added in solution. This might have been anticipated: on the other hand, if carried out in practice it would leave the addition in the hands ot plasterers or helpers and permit considerable opportunity for error.

It was concluded from the above results that a mixture of 10% of aluminium sulphate and a lime of the type of No. 4597 would be a reasonable basis for practical trials of the treatment. Numerous factors must be considered, an important one being, of course, the increase of putty yield, which tends to diminish the cost per unit of wall area. On the other hand, the rate of change of the slope of the curve approaches a maximum at 10%, and with aluminium sulphate at $30.00 per ton and a hydrate cost of $8.00 per ton, the minimum

net increased sales value necessary to cover the material costs alone of a 10% addition would be of the order of $2.20 per ton. It was considered that any cost appreciably higher than this would remove any interest that a lime manufacturer might have.

In order to evaluate the foregoing results in a practical way, arrangements were made for the application of test panels of approximately 24 sq. ft. each in area by a well known local plastering contractor. Mixes of the three types described above were made up and compared with a standard finishing hydrate of high grade from the contractor's warehouse. No instructions were issued to the plasterer other than that he should use the sample according to his customary procedure.

The hydrate used was similar to the three mentioned above and had a workability corresponding to 13 knocks on the flow table. In each case the same proportion of gauging plaster and sand was added and a common backing of standard hardwall plaster was used. The results as observed during application and as subsequently reported by the plasterer through the contractor were as follows:

(a) Hydrate to which aluminium sulphate had been added in solution: This material, possibly owing to inexperience in the addition of large quantities of fairly strong aluminium sulphate solution, tended to be lumpy and was somewhat inferior to the standard magnesian finishing plaster.

(b) Hydrate with which aluminium sulphate was ground to minus 100 mesh: This material was also noticeably inferior to the magnesian hydrate. It showed a slight tendency to "pull" and break. This was probably due to the fine grinding which it had received in the disc pulverizer, for a similar result has been observed with other materials.

(c) Hydrate to which minus 80 mesh aluminium sulphate had been added: This material could not be distinguished from the high grade magnesian hydrate in its behavior under the trowel. The mechanic reported that he would not venture to state that it was at all inferior in "feel."

The four specimens were finished in the same way. All gave very excellent textures; the inferiority noted in the case of *(a)* and *(b)* was manifested only in the slight increase in work required. After a month's aging, no change could be observed in the appearance of the specimens; there was no evidence of the "bloom" which is reported to have been encountered when aluminium sulphate was added to quicklime before hydration.

As a final check on the accuracy with which the flow table indicates the effect of aluminium sulphate additions on the plasticity of the lime, the control magnesian hydrate used in the above-mentioned tests was tested on the flow table. Its rating, according to the standard method, was 25 knocks, which, it will be noted, is of the same order as that of the three mixtures of hydrate plus 10% of aluminium sulphate. It had evidently been duplicated in its properties by the high-calcium lime with addition of aluminium sulphate. It is significant to note that the cost of the magnesian hydrate in this territory is approximately $22.00 per ton.

There was no opportunity to make an accurate estimate of the covering power of these four hydrates during the practical test. The evidence apparently pointed to the magnesian hydrate as being somewhat superior to the others.

In order to have more precise information, "volumes in water" were measured for Sample 7316 plus 10% aluminium sulphate, Sample 7316 untreated, and Samples 4598 and 7315. The last two were high-priced magnesian hydrates. The results are given in Fig. 4 and show a much higher volume in water for the high-calcium lime treated with aluminium sulphate.

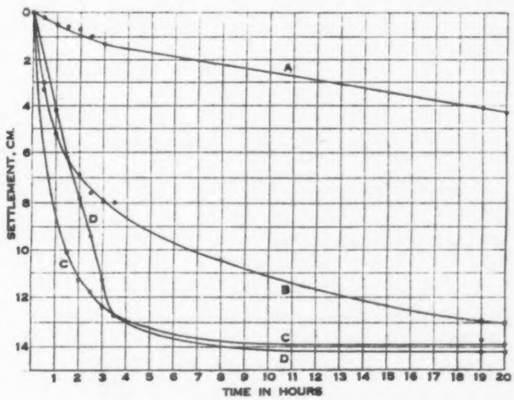


FIG. 4. *Volumes in water of various limes. A, lime No. 7316 plus 10% of aluminium sulphate; B, lime No. 4598; C, lime No. 7315; D, lime No. 7316.*

A further point worthy of note is the increased holding capacity for water which aluminium sulphate additions induce. This is shown in Fig. 5, the data for which were obtained at the same time as those on which Fig. 2 is based. It should be observed that, with more than 10% of aluminium sulphate added in solution, the putty tended to become "sloppy," and therefore undesirable from the point of view of application. Were it not for this fact it is probable that there would have been agreement between Curves A and C in Figs. 2 and 3.

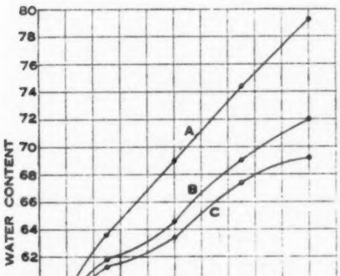


FIG. 5. *Effect of addition of aluminium sulphate on the water-holding capacity of lime (lime No. 4597). A, lime plus aluminium sulphate in solution; B, lime plus sulphate of minus 80 mesh; C, mixture of lime and sulphate ground to minus 100 mesh.*

No definite effect could be ascribed to the aluminium sulphate in relation to setting time or final hardness of the plaster. On the other hand, no deleterious effect is anticipated, as the amount of aluminium hydroxide present is considerably less than 2%. Actually, additions of aluminium hydroxide have been proposed as a means of increasing the final hardness of lime mixtures (5).

It would be reasonable to expect a certain amount of deterioration of lime-aluminium sulphate mixtures in storage, owing to slow reaction and

flocculation of the aluminium hydroxide under dry conditions. To investigate this point, tests were made on mixtures when freshly prepared and after aging in the dry state for a three-week period. The results were as shown in Table II.

TABLE II

DETERIORATION OF MIXTURE DURING STORAGE

Aluminium sulphate mixture		10%	20%
Knock requirement:	Initial	25	33
	After storage	23	30

These results indicate that hydrates containing 10% of aluminium sulphate might be stored for reasonable periods without serious deterioration.

It is considered that these results justify extended commercial trials on the part of certain lime manufacturers whose main tonnage consists of chemical lime or of high-calcium hydrate at present unsuitable for finishing purposes. No great difficulty is anticipated in the addition of the aluminium sulphate, for if it were fed at the pulverizer, adequate mixing without excessive grinding would be assured.

Acknowledgment

The authors wish to acknowledge the co-operation of Mr. M. F. Goudge, of the Department of Mines, Ottawa, who supplied data on plasticity tests with the Emley instrument.

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NOTE ON AN OPTICAL SYSTEM FOR SHADOW RECORDING WITH OSCILLOGRAPHS[1]

By G. A. Woonton[2] and F. W. Pye[3]

Abstract

In this paper is discussed a method of shadow recording for use with reflecting types of oscillographs which simplifies the methods of obtaining time and amplitude rulings on the record. The optical system is rendered portable by the use of a low-intensity light source, compensated by high transmission efficiency. Comparative records indicate that, when used in conjunction with bromide paper, this optical system is of value in recording sinusoidal currents whose frequencies are below 20 cycles per second or currents of complicated wave form whose fundamental frequency is well below 20 cycles per second. No data relative to records on moving film are reported.

Mann (1), in discussing a new portable electrocardiograph, has referred to an optical system of this type. The authors believe that the present paper contains sufficient new information on the details of construction and operation of this optical system to make it of value.

Methods of Oscillograph Recording

Oscillograph records on a moving film or strip of bromide paper are made either with a moving spot or line of light on a dark field, or a moving line shadow on a bright field, depending on the type of oscillograph used. The record is of value for the analysis of the varying currents only when the units (usually e.m.f. against time, or current against time) against which it is plotted, are known, preferably from markings carried on the record itself. Unit markings can be superimposed on the record by periodically varying the light intensity to which the whole film is exposed, both in extension and time; the complexity of the apparatus necessary to bring about such periodic variations in intensity varies with the recording system used.

The bright line or spot method lends itself to recording when the oscillograph element is of the reflecting type. Fig. 1 is a record ol a sinusoidal current obtained in this manner.

This method is wasteful of light flux. If a line of light is used, the line must be generated either by a slit or a cylindrical lens. In the first case all but the small fraction of light flux which passes through the slit is screened out; in the second (since it is not generally convenient to place a cylindrical lens in front of the oscillograph mirror), a broad patch of light floods the mirror plane, but only that portion which is reflected by the mirror reaches the camera. Similar conditions obtain when recording with a spot of light. Intense light sources are required in all cases.

Amplitude rulings are placed on the record by flooding the paper with light of low intensity from a second source, through a cylindrical lens which

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has been etched with millimetre marks in the slit of the camera. Time rulings may be recorded by interrupting the flood lighting beam periodically with a rotating vane, or by allowing a high-intensity beam from a third source to flash periodically on the camera slit, through holes in a rotating disc. Fig. 1 shows amplitude rulings recorded by this method.

The shadow method of recording was originally developed for use with the Einthoven string galvanometer. An Einthoven record obtained in this manner is shown in Fig. 2. A beam of light is passed through the galvanometer in such a manner that a circular patch of light, across which the shadow of the string moves, is developed in the plane of the camera slit. Amplitude rulings on the camera lens furnish recorded amplitude units. A rotating vane interrupts the recording beam periodically and produces time rulings on the paper. One simple optical system and one light source perform the function of the two or three sources and systems required by the other method.

Shadow Recording with Reflecting Oscillographs

Fig. 3 is a schematic diagram of an optical system developed by the authors to make possible the application of the Einthoven type of recording to reflecting oscillographs. Figs. 4*a* and 4*b* are photographs of the optical system in a portable form suitable for general laboratory use.

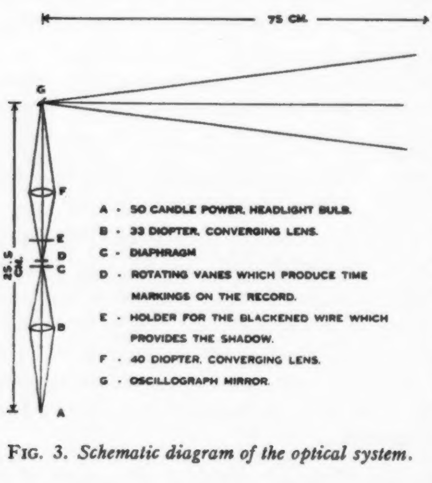


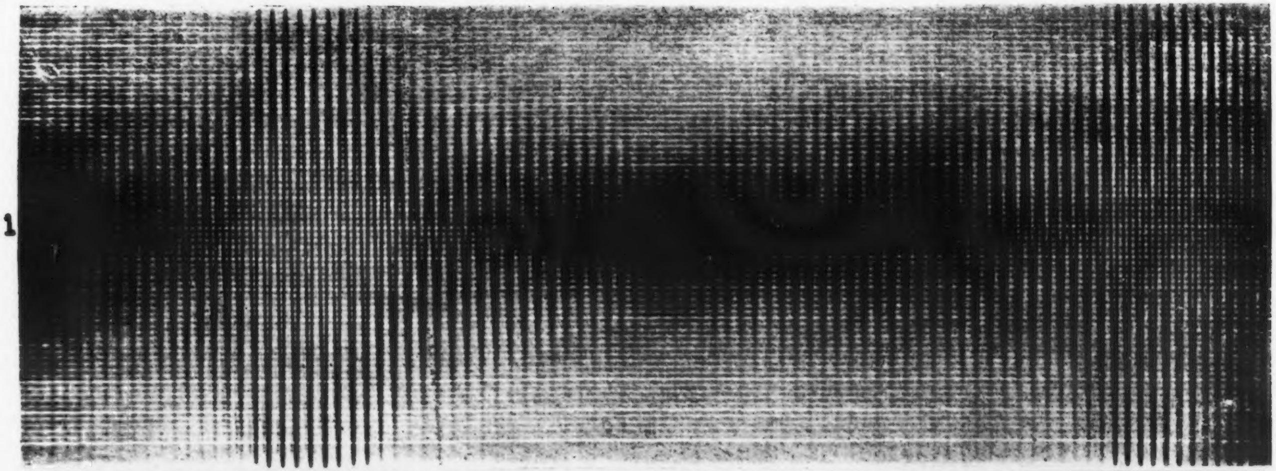
FIG. 3. *Schematic diagram of the optical system.*

The image of the hot filament of a 50 candle power head light bulb is focused on the mirror of an oscillograph by a system of two lenses. The reflected rays diverge from the oscillograph mirror to form a circular spot of light whose diameter is about twice the length of the camera slit. A hairline of blackened wire is located between the two lenses in such a position that a sharp image of the hairline is formed in the plane of the camera slit. The movement of the oscillograph mirror deflects both the image of the hairline and the light spot, at the camera, but since the spot of light is twice as wide as the slit, the slit is always flooded with light regardless of the motion of the hairline.

Amplitude rulings are recorded by the ruled cylindrical lens method. Timing lines are recorded by cutting the light beam periodically at the focal point *D*, by a set of rotating vanes driven by a synchronous motor. In this case the motor carries five vanes, equally spaced, and rotates five times per second. The markings are therefore spaced at intervals of $\frac{1}{25}$ sec. A heavier

PLATE I

1



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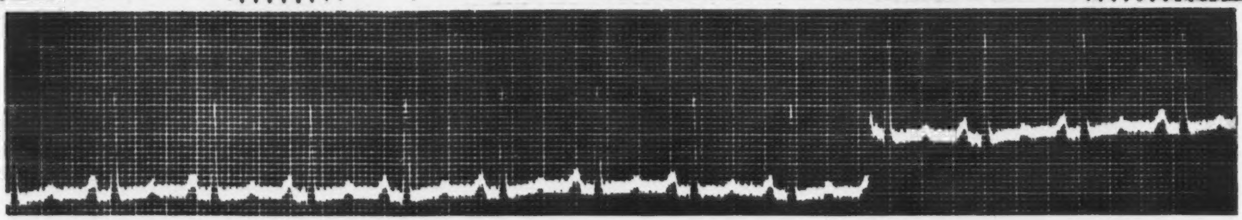

FIG. 1. *Record of a sinusoidal e.m.f., frequency approximately 20 cycles per sec., obtained by the method of a bright line on a dark field.*
FIG. 2. *Cardiogram recorded by the shadow method with an Einthoven galvanometer.*

fifth-second line is obtained by making one of the five vanes wider than the others. A diaphragm at *C*, just behind the rotating vanes, cuts down stray light (due to aberration and other causes) which tends to decrease the contrast of the time graphing.

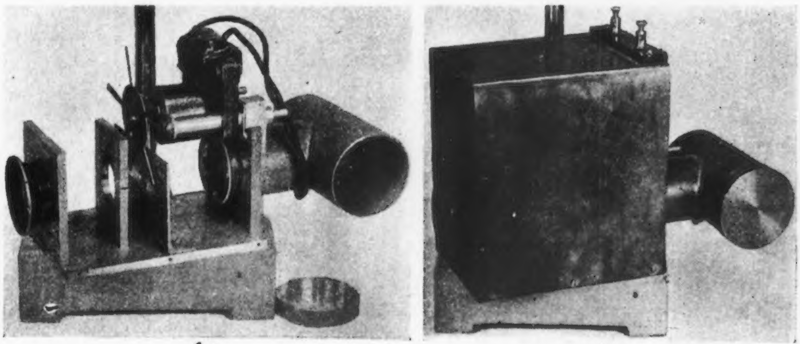

FIG. 4. (a) *The complete optical system with covers removed.* (b) *The optical system with covers in place.*

Frequency Limit of Usefulness

Figs. 5*a*, *b*, *c*, are records made with this system of currents whose frequencies were approximately 4, 10 and 20 cycles per second. Fig. 6 is a cardiogram recorded with a reflecting oscillograph and this system, but without the timing equipment in operation. On the basis of these results it is believed that the system is of value in recording sinusoidal disturbances whose frequencies do not exceed 20 cycles per second, and all types of disturbances whose fundamental is well below 20 cycles per second, even though relatively high harmonics are involved. These data are for contrast grades of bromide paper; no data are at hand regarding the limitations on the system when recording with film.

Precautions

The system discussed above has been designed to operate with a distance of less than 10 cm. between oscillograph mirror and front lens, in order to take full advantage of the possibilities of efficiency and portability associated with the high power lenses used. When the oscillograph is used under conditions of extreme sensitivity and low-frequency response it occasionally picks up a ripple from the magnetic field of the synchronous motor. Magnetic shielding of the optical system or oscillograph will eliminate this source of interference.

The records shown here were made with an optical system whose lenses were not corrected for chromatic aberration; with such lenses the records obtained are sometimes poor. This source of trouble is eliminated by the substitution of achromatic doublets, but their use adds materially to the cost of the system.

If the headlight bulb is operated from an alternating current source, precautions must be taken to prevent synchronization of the vane position with filament current. Synchronization of this kind will cause indistinctness over part, or all, of some of the time marks.

Irregularities in the camera paper speed (such as that caused by a metal rivet in the camera drive belt), give rise on the record to lines that are easily confused with time lines. These ambiguities are avoided by the use of a sewn belt or gears to drive the camera.

Acknowledgment

The writers wish to thank President W. Sherwood Fox and Messrs. Gordon Ingram and A. E. Silverwood of the Board of Governors of this University for financial and other assistance, which has permitted completion of this work as part of a general program intended to facilitate the use of physical equipment in medical research.

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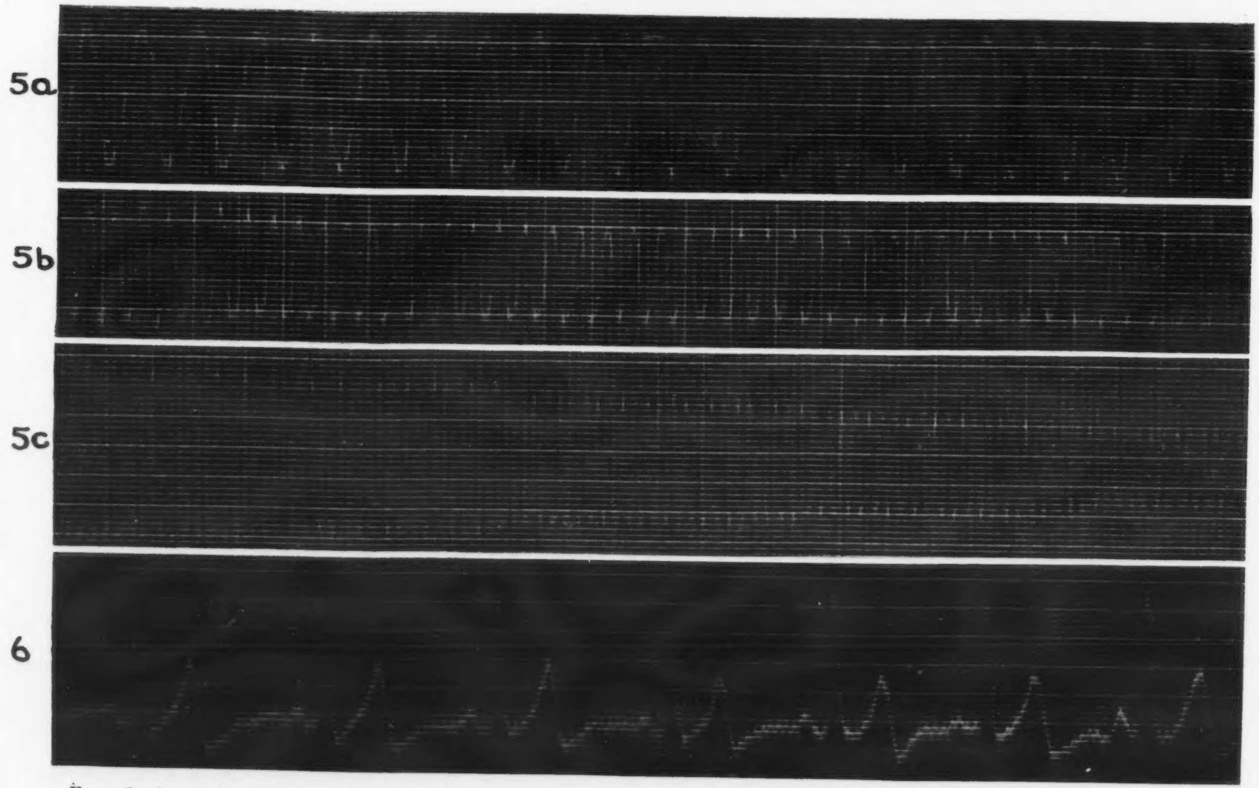

FIGS. 5a, b, c. *Sinusoidal e.m.f.'s recorded by the shadow method in conjunction with a reflecting oscillograph. Frequency—5a, 4 cycles per sec.; 5b, 10 cycles per sec.; 5c, 20 cycles per sec.* FIG. 6. *Cardiogram recorded by the shadow method with a reflecting type of oscillograph.*

MAGNETIC EFFECTS IN SUPERCONDUCTORS[1]

By F. G. A. Tarr[2] and J. O. Wilhelm[3]

Abstract

This paper deals with magnetic effects in metals cooled from above the transition temperature to below the superconducting temperature, while in an applied magnetic field. Previous work of Meissner and Ochsenfeld indicates that at the superconducting temperature the effective permeability of the metal became zero. It is found, however, that the effective permeability is greatly influenced by the composition and geometrical shape of the specimen. There is also, in general, a reduction in flux on removing the magnetic field after the superconducting temperature has been reached, but on re-establishing the field the flux through the specimen remains unchanged.

Introduction

Since the discovery, by Meissner and Ochsenfeld (1), of the permeability changes taking place in a superconductor when it is cooled in a magnetic field, the writers have carried out several experiments with a view to finding out how the results are affected by the composition and geometrical shape of the superconductor. Although this work is not yet complete it seems worth while, at this stage, to collect and present the results so far obtained. The earlier experiments were designed with a view to finding whether there was a limiting particle size below which superconductors would fail to show any change in effective permeability on being cooled from above the transition point down to the superconducting state while in a constant magnetic field. Other experiments on the effect of shape and composition of the specimen suggested themselves as the investigation proceeded.

Experiments

As the work, so far, has not called for any great accuracy, measurements were made using a fluxmeter with stationary search coils as described in a previous paper (3). The magnetic field was produced by two circular coils so arranged, on opposite sides of the cryostat, as to give an approximately parallel field in the region of the specimen under test.

The essential tests made on each superconducting specimen were:—

(a) The determination of the reduction in flux passing through the specimen as it was cooled, in a constant magnetic field, from above the transition temperature to below the superconducting temperature corresponding to the field strength used.

(b) The determination of the reduction in flux passing through the superconductor on removing the external magnetic field directly after process *(a)* had been carried out. Hereafter, for brevity, Test *(a)* will be referred to as ***reduction in flux on cooling*** and Test *(b)* will be referred to as ***reduction in flux on removing field.***

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In many of the specimens tested the search coil windings were not completely shielded by the superconductor which they enclosed, so that when a magnetic field was applied with the specimen at, or below, the superconducting temperature, corresponding to the field strength used, some flux still linked the search coil as indicated by the fluxmeter deflection, and this was taken into account as seen below.

Let T_n be a temperature at which the specimen is completely non-superconducting and T_s be a temperature at which the specimen is completely superconducting for the particular field strength used. If, with the specimen at temperature T_n, an external field is applied, the fluxmeter deflection obtained corresponds to the total flux, ϕ, threading the search coil. Now $\phi = \phi_s + \phi_c$, where ϕ_s is the flux threading the exposed part of the search coil only, and ϕ_c the flux threading the conductor within the search coil. If the specimen is now cooled from T_n to T_s, with no external field applied, and then the same field as above is applied, the deflection obtained corresponds to ϕ_s alone, as no flux can pass through the superconductor in this state. Thus ϕ_c can be calculated. With the specimen again at T_n and the same external field applied, the specimen is now cooled to T_s. The fluxmeter deflection then gives the reduction in flux, ϕ_{rc}, passing through the superconductor. Now, with the specimen still at temperature T_s, the external magnetic field is removed and the deflection gives the reduction in flux, ϕ_r, threading both the superconductor and the exposed part of the search coil. Then $\phi_r = \phi_{rf} + \phi_s$, where ϕ_{rf} is the reduction in flux through the superconductor, and ϕ_s, as before, is the part of the external field threading the exposed part of the search coil only, obtained as outlined above. Thus the reduction in flux in the superconductor, ϕ_{rf}, can be calculated.

For convenience the results are given in percentages as follows:—

Percentage unshielded flux

$$= \frac{\phi_s}{\phi} \times 100\%$$

Percentage shielding

$$= \frac{\phi - \phi_s}{\phi} \times 100\%$$

$$= 100\% - \text{percentage unshielded flux}$$

Percentage reduction in flux on cooling

$$\text{Observed} = \frac{\phi_{rc}}{\phi} \times 100\%$$

$$\text{Corrected} = \frac{\phi_{rc}}{\phi_c} \times 100\% = \left(\frac{\phi_{rc}}{\phi} \Big/ \frac{\phi - \phi_s}{\phi}\right) \times 100\%$$

$$= \frac{\text{Observed percentage reduction in flux on cooling}}{\text{Percentage shielding}}$$

Percentage reduction in flux on removing field

$$\text{Observed} = \frac{\phi_r}{\phi} \times 100\%$$

$$\text{Corrected} = \frac{\phi_{rf}}{\phi_c} \times 100\% = \left(\frac{\phi_r - \phi_s}{\phi} \Big/ \frac{\phi - \phi_s}{\phi}\right) \times 100\%$$

$$= \frac{\text{Observed percentage reduction in flux on removing field} - \text{percentage unshielded flux}}{\text{Percentage shielding}}$$

Of course, corrections made in this way are not strictly accurate (except where all the flux through the superconductor disappears completely on cooling), as it assumes the flux through the unshielded part of the search coil to be constant whereas it actually varies, being greater the less the effective permeability of the superconductor. However, the accuracy of the work did not warrant attempting any more accurate corrections.

In cases where a reduction in flux was found on removing the external magnetic field, as in the above process, it was found that on immediately re-applying the field the deflection of the fluxmeter was exactly the same as if the specimen had been cooled with no external field to the superconducting temperature and a field then applied, *i.e.*, the fluxmeter deflection corresponded to the percentage unshielded flux. In other words this process was not reversible.

Below is given a description of the various specimens tested. The drawings indicate, in addition to the sizes, the positions of the search coils and directions of the applied fields. The field intensities used ranged from 50 to 150 gauss. All specimens were generally of cylindrical shape, either elongated or disc-shaped. In some cases axial, and in others transverse, fields were applied, as indicated by the arrows in the figures.

No. 1. A hollow cylinder of commercial block tin (Fig. 1a).

No. 2. An emulsion of mercury and lard, approximately 50% mercury–50% lard by volume, contained in a glass vessel (Fig. 5). The size of the mercury particles was of the order of 10^{-4} cm. diameter; they appeared under a high power microscope to be surprisingly regular. The emulsion was produced by grinding the mercury into the lard, melting the whole, and then centrifuging while hot.

No. 3. Three pulley-shaped specimens (Fig. 7) made of lead-tin alloy. One was a eutectic (37% lead–63% tin). The other two varied in composition, one being rich in lead (60% lead–40% tin) and the other rich in tin (20% lead–80% tin). All gave the same results.

No. 4. Rose metal (50% bismuth–27.1% lead–22.9% tin) cast round search coil (Fig. 8).

No. 5. Small quartz pebbles embedded in mercury contained in a glass vessel (Fig. 5). Approximately 50% mercury–50% quartz by volume.

No. 6. A solid cylinder of commercial block tin (Fig. 4).

No. 7. A pulley-shaped specimen of commercial block tin (Fig. 7).

No. 8. A pulley-shaped specimen of commercial lead (Fig. 7).

No. 9. Mercury contained in a glass vessel (Fig. 5).

No. 10. A hollow lead cylinder (Fig. 1b).

No. 11. A pulley-shaped specimen of tantalum (Fig. 7).

No. 12. A solid cylinder of commercial block tin (Fig. 6). The search coil was wound in a groove in the cylinder as shown and covered with a strip of tin soldered (with tin) to the cylinder.

No. 13. A solid thin cylinder of commercial block tin (Fig. 2a).

No. 14. A solid thick cylinder of commercial block tin (Fig. 2b).

No. 15. A hollow cylinder of commercial block tin (Fig. 3).

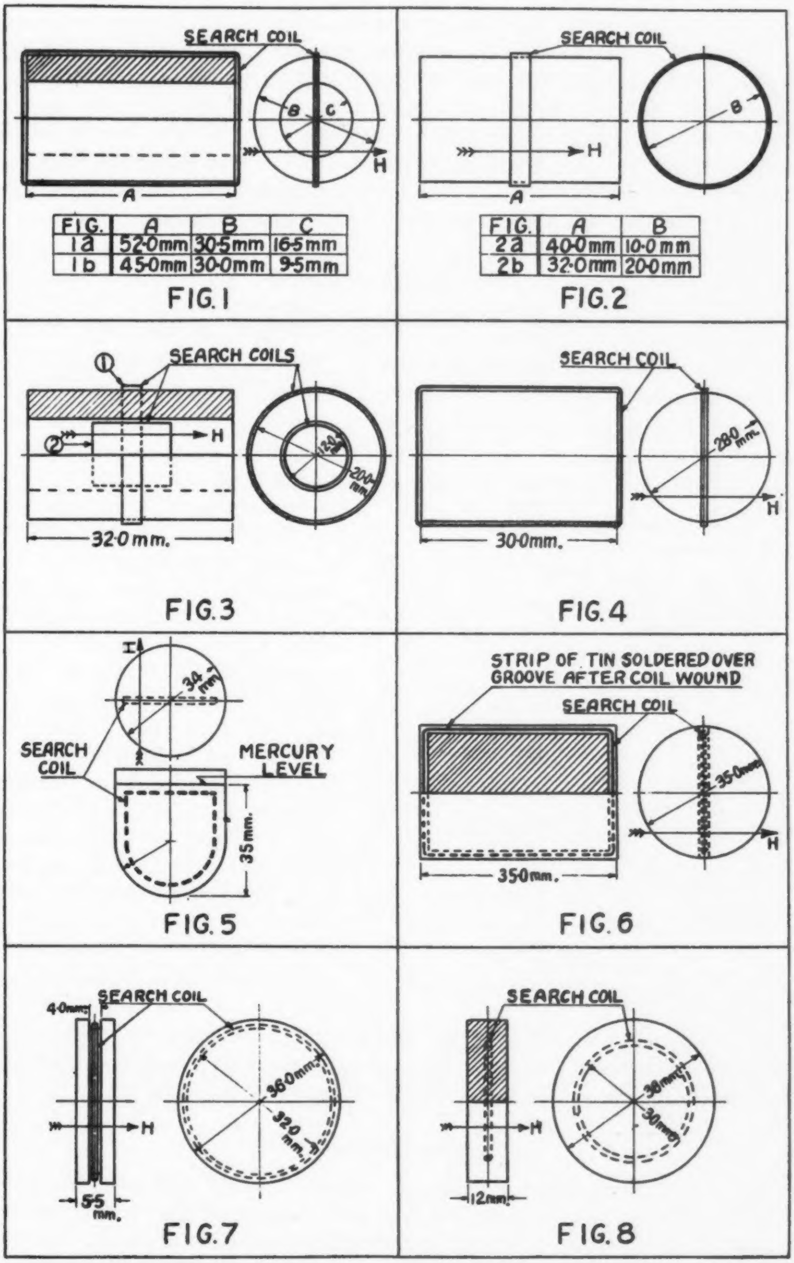
The results of the experiments on these specimens are given in Table I.

TABLE I

MAGNETIC REACTIONS OF SUPERCONDUCTING SPECIMENS

Specimen No.	Fig.	Description	Percentage unshielded flux	Percentage shielding	Percentage reduction in flux on cooling		Percentage reduction in flux on removing field	
					Obs.	Corr.	Obs.	Corr.
1	1a	Hollow tin cylinder	15	85	35	41	—	—
2	5	Mercury and lard emulsion	71	29	29	100	0	0
3	7	Tin-lead alloy pulleys	3	97	0	0	3	0
4	8	Rose metal	0	100	0	0	0	0
5	5	Quartz embedded in mercury	0	100	20	20	30	30
6	4	Solid tin cylinder	12	88	25	28	16	5
7	7	Tin pulley	3	97	10	10	40	38
8	7	Lead pulley	5	95	11	12	29	25
9	5	Mercury	0	100	85	85	15	15
10	1b	Hollow lead cylinder	8	92	60	65	8	0
11	7	Tantalum pulley	7	93	1	1	10	3
12	6	Solid tin cylinder (embedded coil)	0	100	65	65	16	16
13	2a	Solid tin cylinder (thin)	19	81	34	42	45	32
14	2b	Solid tin cylinder (thick)	12	88	24	27	65	60
15	3	Hollow tin cylinder {Coil 1	9	91	24	26	24	16
		Coil 2	0	100	−23	−23	0	0

In most cases repeated tests on the various specimens checked within the limits of experimental error. There was one striking exception, however, in the case of lead pulley (No. 8), where the discrepancies were far beyond possible experimental error. For this experiment, in Table I, are given the average values obtained; the actual corrected values for these tests were 3, 10 and 20% reduction in flux on cooling, and 15, 21 and 39%, respectively, reduction in flux on removing the field. These discrepancies were thought to be due to the way in which the specimen was cooled. As lead becomes



FIGS. 1-8. *Details of specimens referred to in Table I.*

superconducting for the field strengths used above the temperature of liquid helium, the cooling was necessarily carried out in a stream of cold helium gas, so that it was probable that the specimen was not cooled uniformly in each case. In an endeavor to find how the manner in which cooling was carried out affected the results, the hollow lead cylinder (No. 10) was, by the aid of suitable insulation, tested with cooling taking place from either the inside or the outside. In both cases readings of reduction in flux on cooling and on removing the field were taken but no difference was observed.

The only case in which flux passing through the superconductor was completely removed on cooling was that of the mercury and lard emulsion, *i.e.*, the effective permeability of the mercury particles became zero. It will also be seen that only with mercury (No. 9) was the sum of the reductions in flux on cooling and removing the field 100%. The alloys, on the other hand, showed no reduction in flux either on cooling or removing the field.

In addition to the above experiments hysteresis curves were plotted for several of the specimens. Only one curve (Fig. 9) is given as being representative, that of the solid tin cylinder with embedded search coil (No. 12). To obtain this curve the specimen was cooled with no external field to some temperature below the superconducting temperature, but not so low that superconductivity could not be destroyed by the magnetic field at the writers' disposal. The temperature was then kept constant and a magnetic field applied and increased step by step, the fluxmeter reading being noted at each step. After a field sufficient to destroy superconductivity had been applied, the field was lowered step by step, the fluxmeter being read, as before, at each step. The arrows on the curve (Fig. 9) indicate the direction of increasing and decreasing applied field. The curve is plotted to an arbitrary scale. No great accuracy is claimed for this curve as, owing to the time taken, the fluxmeter drift was quite appreciable and corrections had to be made for this.

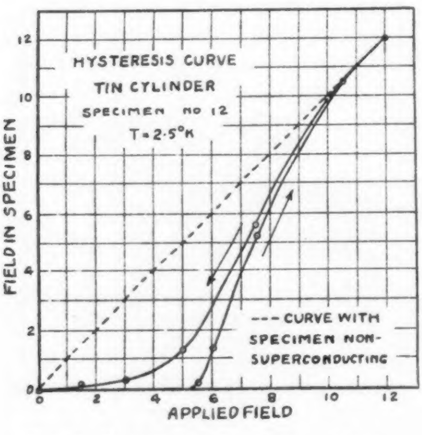


FIG. 9. *Hysteresis curve of a superconductor.*

Conclusion

The original experiments of Meissner and Ochsenfeld indicated that when a superconductor was cooled, in a constant magnetic field, from above the transition temperature to below the superconducting temperature, the flux in the superconductor was either almost or completely annulled. The writers'

experiments show that in general this is not the case, and that there are wide variations in the amount of flux reduction, depending on the shape and composition of the superconductor and on the direction of the applied field. In this the results agree with the result obtained by Mendelssohn and Babbitt in one of their experiments (2) in which they cooled a tin sphere in a constant magnetic field and found, after removing the field, that the sphere had a magnetic moment, although this was hardly to be expected in view of the previous work of Meissner and Ochsenfeld.

Acknowledgment

The authors wish to thank Professor E. F. Burton, Director of this Laboratory, for his encouragement in this work, and also Dr. H. Grayson Smith and Dr. C. Barnes for suggesting several of the experiments recorded herein.

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On a Method of Viewing and Recording Induction Coil Transients with the Cathode-ray Oscillograph

Transient phenomena, in general, cannot be scanned on the cathode-ray oscillograph screen by means of the standard sweep oscillator, because of the non-recurrent nature of the wave and the high velocity of the spot. If the transient is periodic, that part of each wave train which supplies the synchronizing pulse is lost.

The writer's immediate problem lay in the development of apparatus to generate the timing voltage for a cathode-ray oscillograph and in the synchronization of this voltage wave with transients produced by induction coils under the conditions maintained by physiologists when stimulating nervous tissue.

Two methods of stimulation are in common use. Single-shock stimulation involves the production of a single transient in the secondary circuit by a single make or a single break of the primary circuit. Faradic stimulation is produced by periodic transients, generated by periodically interrupting the primary current of the coil either by means of a vibrator or a rotary commutator, which is often fitted with a second set of contacts by which either the make or the break shock may be suppressed by short circuiting the secondary at the appropriate moment. The synchronizing device must be sufficiently flexible to allow retracing of the pattern for primary interruption periods long enough to allow a single transient to be produced and die out completely, and short enough to allow several transients to be partly superimposed if this case ever occurs in practice.

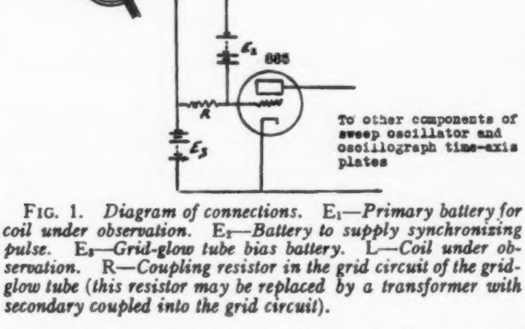
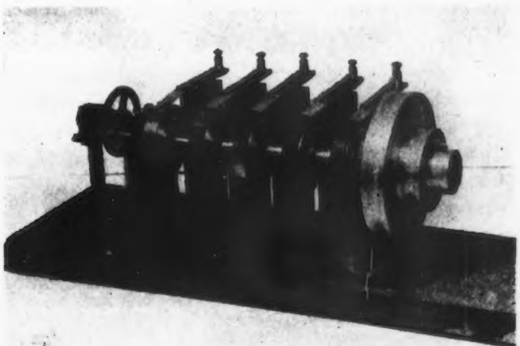


FIG. 1. *Diagram of connections.* E_1—*Primary battery for coil under observation.* E_2—*Battery to supply synchronizing pulse.* E_3—*Grid-glow tube bias battery.* L—*Coil under observation.* R—*Coupling resistor in the grid circuit of the grid-glow tube (this resistor may be replaced by a transformer with secondary coupled into the grid circuit).*

Methods involving relays and more complicated equipment were eliminated for the reasons given above and also because of false transients due to bad arcing at the relay contacts.

Fig. 1 illustrates the circuit and associated equipment developed to overcome these difficulties. It is essentially a combination of the methods of

McFarlane (1) and Turner (2) applied to a sweep oscillator. Fig. 2 is a reproduction of a rotary commutator much like those employed by physiologists and equipped with a synchronizing wheel and contact in addition to those usually employed for making and breaking the primary current and



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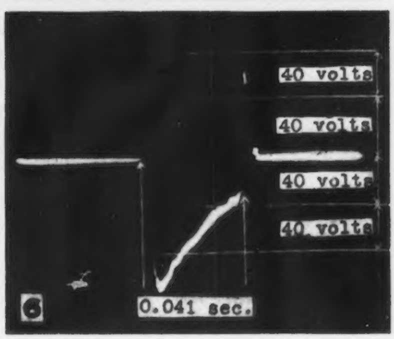

FIG. 2. *The synchronizing commutator. The revolution counter, used in calculating time intervals, is shown geared to the commutator at the left of the photograph.*

FIGS. 3, 4, 6. *Oscillograms obtained from a standard physiological stimulation coil, modified by shunting the interrupter with a condenser, capacity 1 μf.* FIG. 3. *Primary peak current 1.3 amp., iron core, secondary shunted by 75,000 ohms and moved out 10 cm. from position of closest coupling.* FIG. 4. *All conditions as in Fig. 3, except that the secondary was moved out an additional 15 cm.* FIG. 6. *Conditions as in Fig. 3, except secondary in position of closest coupling.*

FIG. 5. *Oscillogram obtained from a standard physiological stimulation coil. In this case the interrupter was not shunted by a condenser. Primary peak current 1.3 amp. iron core, secondary shunted by 1.6 megohms and in closest coupled position.*

suppressing undesired transients in the secondary circuit. The synchronizing wheel presents a bakelite surface to the brush which plays on its periphery except for one very narrow brass contact which occupies only a few minutes of arc of the entire circumference. This strip of brass is connected to a brass disc mounted on the side of the wheel on which a second brush plays. If the two brushes are placed in series with a circuit, the circuit is completed during a very small fraction of a second once in each revolution.

The grid of the grid-glow tube in the sweep oscillator is biased sufficiently negatively relative to the cathode that a discharge cannot take place unless a change in circuit constants occurs. The transformer or coupling resistor in the grid circuit of this tube is coupled in series with an external battery and the brushes of the synchronizing wheel in such a way that the external battery furnishes a pulse which neutralizes the negative bias on the tube, and allows a discharge to take place each time that contact is made at the synchronizing wheel. After each discharge the grid of the tube again takes control, and since the synchronizing contact has been broken as the commutator turns, a second oscillation cannot occur until the contact has completed one revolution and returned to its previous position. Since by this means the transient appears at a definite time after the spot starts its sweep across the screen, perfect retracing is possible. The transient wave may be spread across the screen, or condensed to any degree since the velocity of the spot is entirely independent of the periodicity of the sweep. Amplitude and frequency of the sweep oscillator are controlled in the usual manner. Figs. 3, 4, 5 and 6 are photographs of wave patterns produced on the oscillograph screen by induction coil discharges, controlled by the method just discussed.

Graphical analyses of transient patterns require a determination of the units on both the time and voltage axis. Voltage units may be easily determined by applying known voltages to the deflecting plates after the transient record is made. The exposed film shows these further records as graph lines. Some experimenters have obtained time units by superimposing a sine wave of known frequency on the record. The writer has found it more convenient to calculate the time interval between the start of the make and break transients directly from the film by means of data regarding the angular velocity of the commutator, furnished by a permanently attached revolution counter, and the known angular separation between two ends of the primary contact segment. In cases where either the make or the break transient is to be viewed alone, an extra contact wheel is used to supply a peak on the screen at a definite number of degrees of arc before the transient appears. As before, data sufficient to determine the time scale completely can be obtained from the film by a simple measurement of separation between the two impulses.

The various contact wheels of the commutator are each held in place by a single set screw. The position of the pattern on the screen can be readily adjusted by changing the angular position of the various contacts relative to one another. Allowance can also be made, by the same method, for phase changes if the e.m.f. under investigation is to be amplified or attenuated.

In the first experiments with this device, difficulties in synchronizing were discovered owing to impulses induced by the coils in the synchronizing network. It was found that these difficulties could be avoided by biasing the grid-glow tube far beyond the threshold of ignition reached by these false impulses, and counterbalancing the high bias (sometimes 90 volts) by a correspondingly high-potential battery in the synchronizing circuit.

The writer wishes to express his thanks to Professor F. R. Miller for suggesting this problem and for his help and suggestions regarding the physiological problems involved, and to Mr. F. W. Pye for his help in designing and constructing the commutator.

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Received October 22, 1934.